The Structure of Filipin

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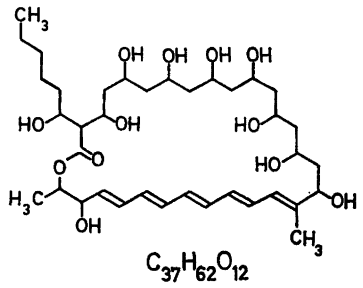
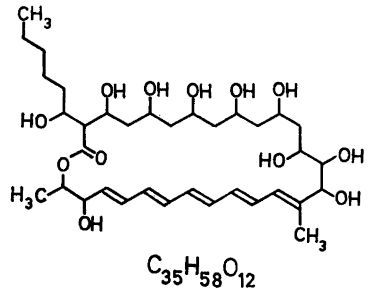
The polyene antibiotic filipin was recently assigned structure 1.[1] The calculation of the proposed empirical formula, $C_{37}H_{62}O_{12}$,



$C_{37}H_{62}O_{12}$

1

was based on a careful proton-count in the nuclear magnetic resonance spectrum of the non-crystalline "peracetyl filipin".



$C_{35}H_{58}O_{12}$

2

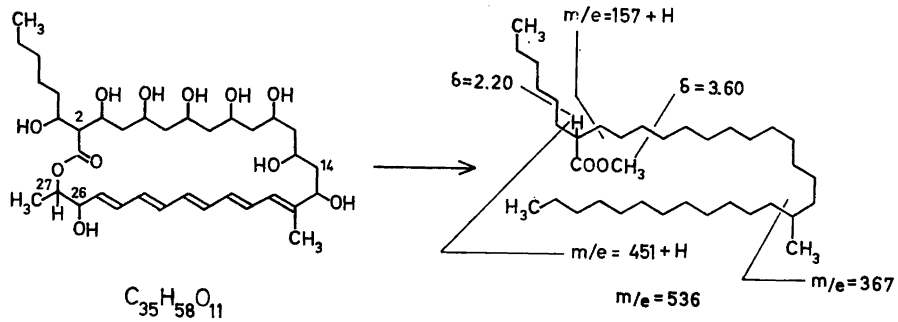
However, the similarity of the chemical and physical properties of filipin and lagosin, 2, particularly the close agreement of the extinction coefficients of the pentaene chromophores, has led to the proposal that the empirical formula of filipin is rather $C_{35}H_{58}O_{11}$.[2] The biogenetically reasonable structure, 3, lacking the hydroxyl group at C-14, was then tentatively suggested.[2]

It is of interest to note that fungichromin [3] possesses a gross structure identical with that of lagosin. As these two antibiotics show a small but definite difference in specific rotation and display slightly different rotatory dispersion curves, the difference may be of a configurational or conformational nature. The structure of fungichromin was supported by reductive degradation of the antibiotic to 7,21-dimethyltritriacontane, 4, whose structure was established by mass spectrometry and by synthesis.

Since both the nuclear magnetic resonance method and the ultraviolet measurements seem to lack the accuracy required to distinguish unambiguously between $C_{37}H_{62}O_{12}$ and $C_{35}H_{58}O_{11}$, or a close homologue, another approach appeared necessary to prove the structure of filipin beyond doubt.

Catalytic hydrogenation of filipin in glacial acetic acid at 280° and 212 atm[4] gave an oily product which after methylation showed the properties (thin layer and gas chromatography, mass and nuclear magnetic resonance spectroscopy) of the methyl ester of a saturated C_{35}-monocarboxylic acid, containing small amounts of the 1,4 or 1,5 epoxy derivative. After treatment of the mixture with hydroiodic acid–red phosphorus, followed by methylation and catalytic hydrogenation, a gas chromatographically pure product was obtained. By mass spectrometry the molecular weight was found to be 536, which corresponds to $C_{34}H_{69} \cdot COOCH_3$. The fragmentation pattern agreed with structure 5. The nuclear magnetic resonance spectrum of the ester displayed a three-proton singlet at $\delta = 3.60$ and a broad one-proton absorption at $\delta = 2.20$. Oxidation of the methyl ester with chromium trioxide in sulfuric acid followed by gas chromatographic analysis of the esterified steam volatile acids proved the presence of large amounts of heptanoic and tridecanoic acid, but no higher homologues could be found.

Reduction of 5 with lithium aluminum hydride, treatment of the resulting alcohol with hydroiodic acid–red phosphorus, and finally catalytic hydrogenation produced a saturated hydrocarbon which was gas chromatographically homogeneous. Its molecular weight, 492, as determined by mass spectrometry, corresponds to $C_{35}H_{72}$. The fragmentation pattern was identical with that displayed by synthetic 7,21-dimethyltritriacontane, 4, which thus represents



$C_{35}H_{58}O_{11}$

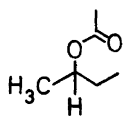
3

5

4

the carbon skeleton of filipin. The same hydrocarbon was also obtained from fungichromin.

Earlier it had been demonstrated that filipin is inert to periodate under neutral conditions, but after opening of the lactone ring with base it consumes one mole.[1] Acetaldehyde was formed and the lactone is therefore closed at C-26 or C-27. It has now been found that the nuclear magnetic resonance spectrum of filipin in pyridine displays a one-proton quartet with additional fine structure at $\delta = 5.30$. The chemical shift and splitting pattern



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are characteristic for a methine proton as shown in partial structure 6.[4,5] This finding strongly indicates, that the hydroxyl group at C-27 is lactonized and that filipin therefore contains a twenty-eight membered lactone ring.

The nuclear magnetic resonance spectrum of filipin in pyridine shows a three-proton singlet at $\delta = 2.02$ due to a methyl group attached to a doubly bonded carbon atom (*cf.* Ref.[1], p. 387). Between $\delta = 2.0$ and 2.5 no further absorption could be detected; filipin therefore does not contain any methylene protons adjacent to a carbonyl group or a carbon-carbon double bond. The structure of the C_6-side chain and the presence and position of the pentaene system have been demonstrated earlier.[1]

Since oxidation of the antibiotic with chromium trioxide in sulfuric acid under forcing conditions did not yield succinic acid or any of its higher homologues (*cf.*, however, Ref.[6]), it is concluded that filipin possesses structure 3.

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Diphenylpicrylhydrazyl as a Reagent for Terpenes and Other Substances in Thin-Layer Chromatography

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Diphenylpicrylhydrazyl (DPPH) is a free radical of purple colour, relatively stable even in solution. It reacts with other radical species, and it has been used as a scavenger for radicals, especially for those produced in high-energy radiation. In the presence of air, DPPH reacts as a hydrogen acceptor with a variety of ethylenic compounds and phenols. The kinetics and mechanism of these reactions have been investigated by several workers.[1-4] During the reaction the purple colour changes to light yellow. This suggests DPPH as a "reversed" reagent for thin-layer chromatography. Its use as a reagent for phenols in paper chromatography has, as a matter of fact, already been suggested.[6]

DPPH was found to react with terpenes, and this note will describe its use as a sensitive visualizer for these compounds after separation by thin-layer chromatography.

Experimental. The substances to be tested have been run on silica-gypsum (13 %) thin-layer plates, dried 30 min at 110°C, and kept in an exsiccator until used. The eluating solvent was a mixture of chloroform and benzene (1:1 v/v). Approximately 100 μg of each terpene were applied to the plates. The solvent front was allowed to ascend 10 cm. After evaporation of the solvent, the plates were immediately sprayed with a solution of DPPH (Fluka, Switzerland) in chloroform (15 mg in 25 ml). The plates were then rapidly oven-heated to 110°C and kept at this temperature for 5—10 min. A positive reaction, indicating the presence of a substance reacting with DPPH, gives a yellow spot on the purple background.

Table 1.

Substances	No. of double bonds	Structure C = Cyclic AC = Acyclic	Reaction
Hydrocarbons			
Myrcene	3	AC	M
allo-Ocimene	3	AC	M
p-Cymene	3	C	W
Limonene	2	C	M
α-Phellandrene	2	C	M
β-Phellandrene	2	C	M
α-Terpinene	2	C	M
γ-Terpinene	2	C	M
Sabinene	1	C	M
α-Pinene	1	C	W
β-Pinene	1	C	W
Camphene	1	C	W
Δ^3-Carene	1	C	S
Santene	1	C	S
Fenchene	0	C	M
Humulene	3	C	S
Caryophyllene	2	C	S
Cedrene	1	C	M
Alcohols			
Linalool	2	AC	S
Lavandulol	2	AC	S
Menthol	0	C	M
4-Terpinenol	1	C	S
α-Terpineol	1	C	M
Isopulegol	1	C	S
Carveol	2	C	S
Sabinol	1	C	S
Borneol	0	C	M
Isoborneol	0	C	S
Thujylalcohol	0	C	M
Fenchylalcohol	0	C	S
neo-Isothujyl-alcohol	0	C	M