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THE STRUCTURE OF TETRENOLIN

A NEW ANTIBIOTIC SUBSTANCE

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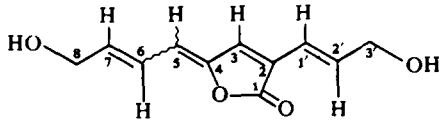
Abstract—Tetrenolin, a new antibiotic substance, produced by *Micropolyspora venezuelensis*, was shown to be the γ-lactone of 2-(3-hydroxy-1-propen)-4,8-dihydroxy-2,4,6-octatrienoic acid (I). The structure determination was based on spectroscopic studies of I and of its octahydroderivative II.

TETRENOLIN is a metabolic product isolated from cultures of *Micropolyspora venezuelensis* Thiemann 1969, provided with an *in vitro* activity against Gram-positive bacteria.[1] The product was recovered from the fermentation broth by extraction with n-butanol and obtained in a pure state by successive crystallizations from chloroform. Tetrenolin proved to be the γ-lactone of 2-(3-hydroxy-1-propen)-4,8-dihydroxy-2,4,6-octatrienoic acid (I) on the basis of the evidences reported below.



(I)

Tetrenolin is a yellow crystalline substance, m.p. 126–128°. Elemental analysis, $C_{11}H_{12}O_4$ (MW 208, 22), confirmed by the M^+ peak of the mass spectrum (Fig. 1). The IR spectrum is consistent with the presence of OH groups (3200 cm^{-1}), HC═CH bonds (3040, 1650, 1630 and 1620 cm^{-1}) and a 5-membered ring CO group (1760 and

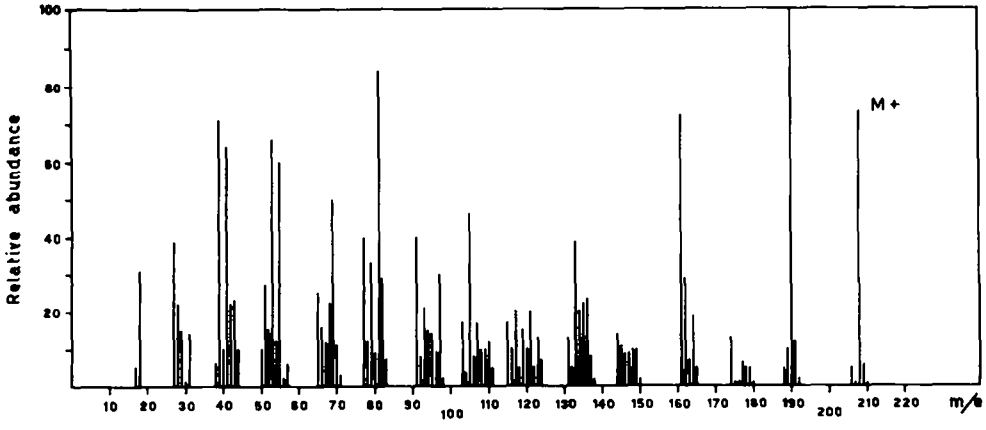


FIG. 1 Mass spectrum of tetrenolin

1740 cm^{-1}). Typical IR aromatic absorptions are not present, in accordance with the general appearance of the mass spectrum. The UV spectrum in MeOH shows maxima at 208 mμ (ε, 12·300) and at 340 mμ (ε, 42·900), suggesting an extended conjugation. The PMR spectrum in DMSO-d_6 shows the presence of two CH_2—OH groups and of six hydrogens on conjugated double bonds. The two CH_2OH groups (CH_2: m centered at 5·79 τ; OH: tr at 4·95 τ,[2] disappearing by shaking with D_2O) are adjacent to a double bond, as derived from the paramagnetic shift of the CH_2 signal with respect to the reported chemical shift of a $-\overset{|}{\underset{|}{C}}-CH_2-O$ group.[3,*]

One of the hydrogens on a conjugated double bond is a singlet at 2·29 τ, a chemical shift which suggests that its position is β on a double bond conjugated with a CO group.[4]

The relative position of the H atoms could be determined by PMR spin-decoupling experiments in pyridine-d_5 solution, a solvent which spreads the signals over a wider range. As shown in Fig. 2, the excitation, at two different frequencies, of the methylene multiplets established that the methylene at 5·42 τ is α to the hydrogen at 3·55 τ and β to the hydrogen at 2·80 τ and that the methylene at 5·38 τ is α to the hydrogen at 2·62 τ and β to the hydrogen at 3·10 τ. By excitation of the doublet at 3·88 τ, the corresponding hydrogen was shown to be adjacent to the hydrogen at 2·80 τ. Spin-decoupling experiments were performed also on the olefinic hydrogens, confirming the assignments. Thus, the moieties $HO-CH_2-CH=CH-CH=C\diagdown$ and $HO-CH_2-CH=CH-\overset{|}{C}=$ are present in the molecule. From the fact that the remaining hydrogen (s at 2·55 τ) appears to be in β-position to a CO group, as previously assessed, the moiety $-CH=\overset{|}{C}-C\overset{O}{\diagdown}$ could be postulated. Since the molecule has eleven C atoms, in this moiety the C atom α to the CO has to coincide with the terminal one of the 4-carbon-atoms moiety. Therefore, the only consistent way to arrange the three moieties together with the remaining O atom is by formulating the 5-membered lactone ring of formula I. The IR frequency of the CO group is determined by the two opposite and compensating conjugative and vinylic effects operating on it. The UV absorption maximum wavelength (340 mμ) is in accordance with the value calculated for I adopting the rule for a conjugated ketone chromophore.[5] In the mass spectrum (Fig. 1) the relative abundance of the M^+ peak is in agreement with the polyene structure. The loss of water (m/e = 190) is due to the presence of alcoholic groups.

By catalytic reduction tetrenolin yielded an octahydroderivative (II) whose IR spectrum shows substantially unchanged CO absorptions (1750 and 1735 cm^{-1}). This is only compatible with the presence of a 5-membered lactone, whose CO frequency is determined in I by the two opposite and compensating effects removed in II by hydrogenation. In the PMR spectrum of II ($CDCl_3$) the broad signals at 7·40 τ, corresponding to a methyne adjacent to a CO group, and at 5·65 τ, corresponding to a methyne bearing an oxygen atom, confirm the presence of the α and γ substituted lactone.

* The chemical shift of a methylene group is substantially the same in DMSO-d_6 and in $CDCl_3$.

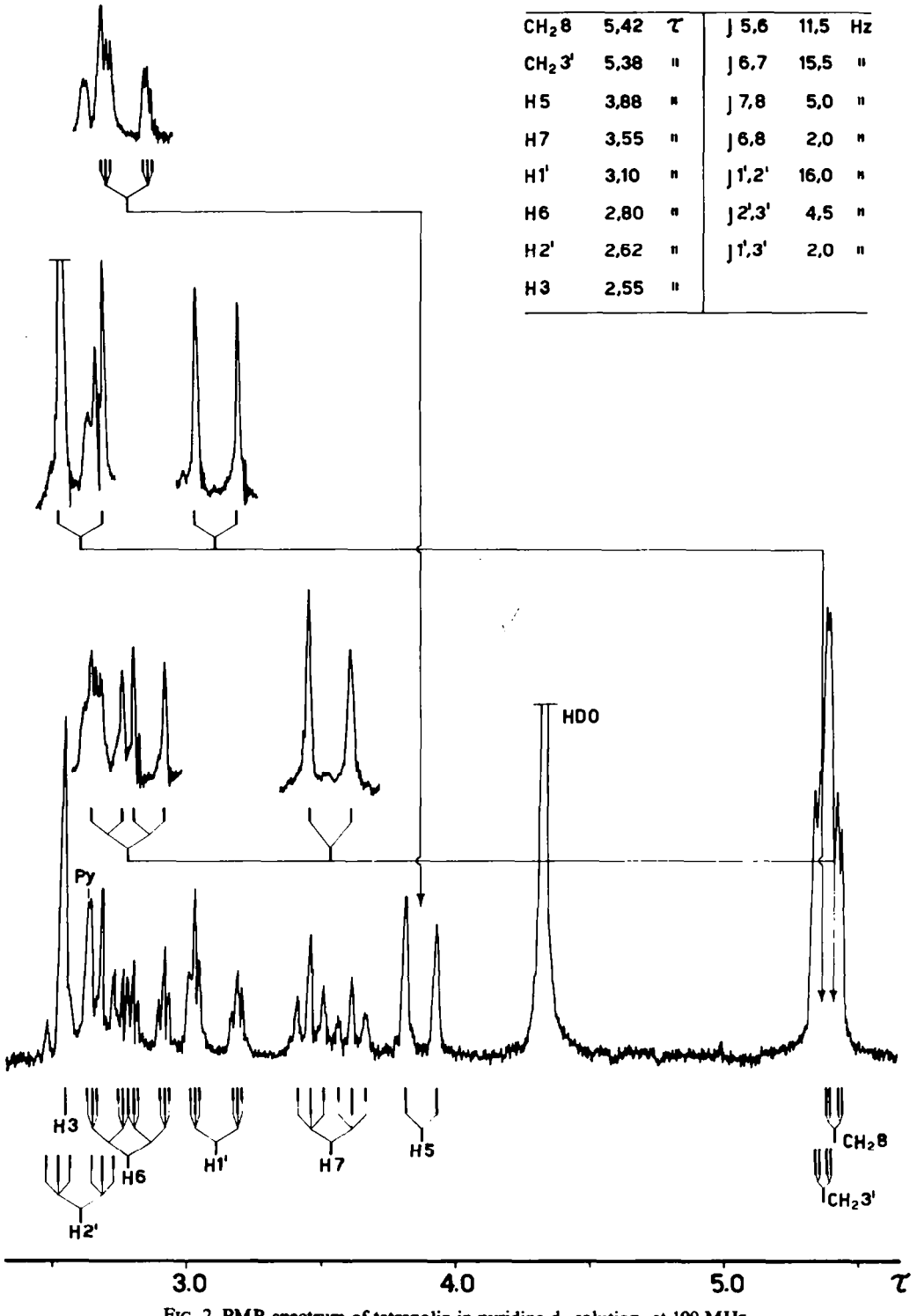


FIG. 2 PMR spectrum of tetrenolin in pyridine-d_5 solution, at 100 MHz

The configuration of tetrenolin was derived on the basis of the coupling constants obtained from the PMR spectrum of I in pyridine-d_5 (Fig. 2). The values of $J_{6,7}$ and $J_{1',2'}$ indicate the *trans* configuration for the corresponding double bonds. Concerning carbon-5, the configuration cannot be established with the PMR technique, since the two possible orientations of the hydrogen at C-5 correspond to the two non-W patterns for which coupling constants of the same order of magnitude have been reported.[6]

The α,β-butenolide moiety has been found in the metabolites of several microorganisms,[7] as for example in tetronic acid derivatives, some of them displaying antibacterial activity.[8,9] Considering the compounds biogenetically related to tetronic acid,[10] the occurrence of 2,4-substituted butenolides appears rather common, whereas the extended conjugated system of tetrenolin is, to our knowledge, completely new.

EXPERIMENTAL

IR spectra were run on a Perkin–Elmer mod. 125 grating spectrophotometer as nujol mulls. PMR spectra were taken with a Varian A-60 (60 MHz) or with a Varian HA-100 (100 MHz) spectrometer in the solvents indicated (5–10% w/v), with TMS as internal reference (τ = 1000 ppm). The mass spectrum was recorded with a Hitachi Perkin–Elmer RMU-6D spectrometer (single focus) at electron ionizing voltage 70 V. UV spectra were taken with a Unicam SP800 spectrophotometer.

Isolation of tetrenolin (I). The product was isolated from cultures of *M. venezuelensis* as described.[1] Pure tetrenolin was obtained by crystallization from $CHCl_3$, m.p. 126–128°, R_f = 0·5 (TLC $CHCl_3$/MeOH 9/1). (Found: C, 62·9; H, 5·9; O, 31·2; $C_{11}H_{12}O_4$ requires: C, 63·4; H, 5·8; O, 30·8%).

Octahydrotetrenolin (II). A soln of I (0·5 g) in MeOH (100 ml) was reduced at normal press with H_2 over 5% Pd/C catalyst (0·1 g). The soln, filtered from the catalyst, was evaporated under reduced press to yield a colourless oily residue. Chromatography over silica-gel in $CHCl_3$/MeOH (95/5) yielded, besides small impurities, a colourless oil R_f = 0·6 (TLC) that was not analyzed and a white solid (150 mg) R_f = 0·3 (TLC). The solid product (II) was crystallized from $CHCl_3$, m.p. 60–62°. (Found: C, 61·0; H, 9·4; O, 29·3; $C_{11}H_{20}O_4$ requires: C, 61·1; H, 9·3; O, 29·6%).

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