THE STRUCTURE OF TETRACENOMYCIN C

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(Received for publication December 16, 1991)

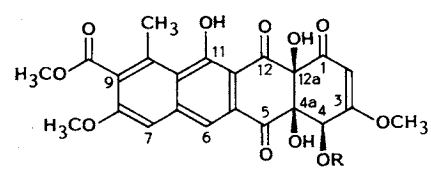
Tetracenomycin C (**1**), a new antibiotic produced by *Streptomyces glaucescens* (strain Tü 49) is structurally related to the anthracyclinones[1,2]. It was the first example of a discrete group of antibiotics whose members show biological activity mainly against Gram-positive bacteria of the genus *Streptomyces* and a moderate cytotoxic effect on L1210 leukemia cells *in vitro* (IC_{50} 1.2 μg/ml) caused by an interaction with DNA. In the meantime **1** has also been isolated by a Chinese group[3]. Similar antibiotics, the elloramycins[4~6] and tetracenomycin X[7], have been described and the first structure-activity relationships have been investigated within this group[8].

The constitution of tetracenomycin C, $C_{23}H_{20}O_{11}$, was established by chemical and spectroscopic methods[1,2,4]. In this paper we wish to report the X-ray analysis of **1** leading to the relative configuration of the three stereocenters. The application of the HELMCHEN method[9] for secondary alcohols allowed us to establish the absolute configuration at C-4 and thus to determine the correct enantiomer.

Crystals of **1** (yellow needles) were obtained from methanol. The crystal data for **1**·0.5 MeOH are as follows: Orthorhombic, space group $P2_12_12_1$, $a=1{,}498.2$ (2), $b=2{,}023.6$ (3), $c=1{,}463.6$ (2) ppm, $U=4.437\,nm^3$, $Z=8$, $D_{calc}=1.462\,g\cdot cm^{-3}$; 4,852 unique intensities measured, $2\theta_{max}=60°$ (Mo-Kα radiation), structure solved by Patterson search techniques using a calculated search fragment with 25 atoms[10], H atoms located by difference electron-density determination and refined with a riding model, anisotropic refinement of C and O atoms with SHELXTL based on 3,003 reflections with $|F|>3\sigma(F)$, $R=0.081$ ($R_w=0.074$). Further details of the crystal structure investigation are available on request from the Fachinformationszentrum Energie, Physik, Mathematik GmbH, D-7514 Eggenstein-Leopoldshafen 2 (FRG), on quoting the depository number CSD-55985, the names of the authors and the journal citation.

The three hydroxy groups at C-4, C-4a and C-12a are *cis* to each other (Fig. 1). The last two are involved in intramolecular hydrogen bonds to the carbonyl oxygen atoms at C-5 and C-1, respectively, in addition to the expected interaction between 11-OH and the carbonyl oxygen atom at C-12. The rings A and B form half-chair conformations with C-4a and C-12a deviating from the respective mean planes to opposite sides. The carboxymethyl group at C-9 is almost perpendicular to the planar rings C and D; however, the two independent molecules in the crystal differ in the sign of the dihedral angles. This kind of isomerization induced by the neighboring methyl and methoxy groups is similar to the one observed by NICOLAOU *et al.*[11]. The molecules form infinite stacks with rings C and D onto each other. The crystal packing is stabilized by intermolecular hydrogen bonds with participation of methanol molecules.

A solution of **1** (67 mg) in trifluoroacetic anhydride (10 ml) was treated with 163 mg of [a] (*R*/*S*)-2-phenylbutyric acid and [b] (−)-(*R*)-2-phenylbutyric acid for a period of 4 hours at 40°C and overnight at room temperature. The solvent was evaporated, the residue dissolved in aqueous methanol to hydrolyze the trifluoroacetoxy groups and the solution was then evaporated to dryness. Trifluoroacetic anhydride directed the acylation with 2-phenylbutyric acid towards 4-OH by temporarily protecting the other hydroxy groups. The reaction



1 R=H

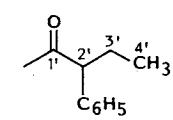
2 R= 

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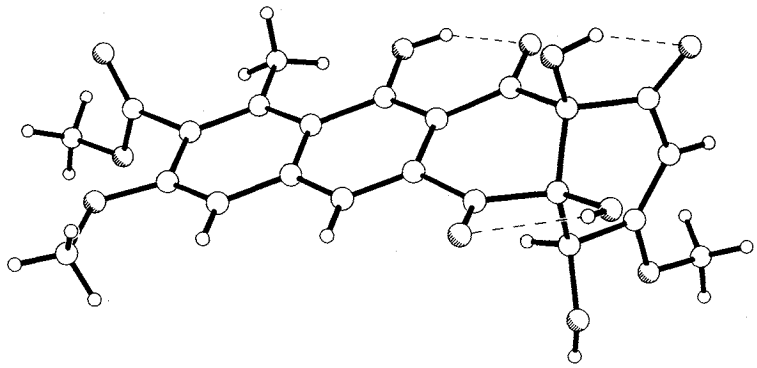
Fig. 1. Perspective view of **1** (O atoms shaded) with the O–H · · · O hydrogen bonds indicated by broken lines.



mixture was chromatographed on silica gel (plates, 20 × 40 cm, $CHCl_3$ - MeOH, 85 : 15). In addition to 12 mg of **1**, about 20 mg of 4-*O*-(2-phenylbutyryl)-tetracenomycin C (**2**) were isolated from the main yellow zone which was further purified on Sephadex LH-20 (column: 100 × 2.5 cm, MeOH). **2** was obtained as amorphous powder: Rf 0.78 (TLC plates, Macherey-Nagel Sil G/UV 254 + 366, 0.25 mm silica gel on glass, $CHCl_3$ - MeOH, 9 : 1; **1**: Rf (0.11); IR (KBr) 1740, 1710, 1680, 1609 cm^{-1}; EI-MS (70 eV) *m/z* (abundance) 618 (2%, M^+, HR calcd for $C_{33}H_{30}O_{12}$ and found: 618.1737), 587 (1%, M − 31), 505 (1%), 454 (3%), 438 (1%), 410 (1%), 359 (18%), 327 (3%), 232 (1%), 164 (21%), 119 (70%), 91 (100%). The position of the 2-phenylbutyryl residue was confirmed by the significant low-field shift of 4-H in the ^1H NMR spectrum of **2** (δ 6.13, $J = 2$ Hz) compared with 4-H of **1** ($\Delta\delta$ 1.23 ppm). Using racemic 2-phenylbutyric acid the isolated diastereomeric mixture of **2** showed clearly separated singlets for the methoxy group at C-3 (δ 3.49/3.75, $\Delta\delta$ 0.26 ppm) and for 6-H (δ 7.58/7.83, $\Delta\delta$ 0.25 ppm). Smaller effects could be seen for 2-H (δ 5.61/5.68, $\Delta\delta$ 0.07 ppm) and 7-H (δ 7.04/7.08, $\Delta\delta$ 0.04 ppm). The remaining signals of **2** appeared in the expected regions showing no shift effects: δ 0.87 (t, $J = 6$ Hz, 4′-H_3), 1.85 (m, 3′-H_2), 2.83 (s, 10-CH_3), 3.50 (t, $J = 6$ Hz, 2′-H), 3.95 (s, 8-OCH_3), 3.98 (s, 9-$COOCH_3$), 7.25 (m, 5H, phenyl group), 13.50 (s, 11-OH).

Using (−)-(*R*)-2-phenylbutyric acid the isolated **2** showed only one signal set for the protons near C-4. An upfield shift was observed for 6-H (δ 7.58) and 7-H (δ 7.04), while 3-OCH_3 (δ 3.71) and 2-H (δ 5.68) had the normal position. According to the rules of HELMCHEN[9], the configuration at C-4 can be deduced to be *R*. Taking into account the results from the X-ray analysis, the absolute configuration of tetracenomycin C can now be described as 4*R*, 4*aR*, 12*aR* (Scheme 1 and Fig. 1). This result is in agreement with that derived from elloramycin by chemical transformation of the aglycone into the tetramethyl ether of **1**[4].

Acknowledgment

We are grateful to Professor Dr. H. ZÄHNER, Institut für Biologie II der Universität Tübingen (FRG), for providing us with tetracenomycin C. This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

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