ISOLATION, CHARACTERIZATION, AND STRUCTURE OF RABELOMYCIN, A NEW ANTIBIOTIC

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Rabelomycin, a new benz [*a*] anthraquinone antibiotic, has been isolated from fermentation broths of *Streptomyces olivaceus* ATCC 21,549. It is active against gram-positive microorganisms. Rabelomycin loses water easily when treated with acid. Structures I and III have been proposed for rabelomycin and its dehydration product, respectively.

Rabelomycin, a new antibiotic, is produced by a strain of *Streptomyces olivaceus* ATCC 21,549 isolated from a soil sample taken at Jean-Rabel, Haiti. Structure I has been assigned to rabelomycin on the basis of spectral and chemical studies of the crystalline material.

Fermentation

Streptomyces olivaceus ATCC 21,549 was isolated from soil by means of the bioautographic technique previously described[1]. The culture was maintained by storage in liquid nitrogen and, when needed, was grown out on tomato paste-oatmeal agar slants. This medium is made by adding one volume of a boiling aqueous suspension of tomato paste (4 %) and oatmeal (4 %) to one volume of a boiling aqueous solution of agar (3 %).

The growth from well-sporulated slants was suspended in 0.01 % sodium lauryl sulfate and used to inoculate the germinator medium, which had the following composition:

Soybean meal (Staley's 4S)	15.0 g	$CoCl_2 \cdot 2H_2O$	0.005 g
Dehydrated mashed potato	15.0	$CaCO_3$	10.0
Glucose	50.0	Agar	2.5

Distilled water to 1,000 ml

After 96 hours of incubation at 25°C on a rotary shaker, the growth was used to provide inoculum for fermentation vessels containing the same medium, except that the concentrations of soybean meal and glucose per 1,000 ml were 30.0 and 15.0 g, respectively. The fermentation was harvested after 72~96 hours at 25°C.

The progress of the fermentation, as well as the subsequent steps during isolation, was followed by several bioassay techniques with *Staphylococcus aureus* FDA 209P as the indicator organism: twofold tube dilution assay, paper disc-agar diffusion assay, and bioautography of thin-layer chromatograms. Two solvent systems for thin-layer

chromatography on silica gel were normally employed: a) chloroform - methanol - piperidine (94 : 5 : 1 by volume) and b) benzene - methanol (9 : 1 by volume). Rabelomycin had an Rf of 0.4 in the first system and of 0.5 in the second.

Isolation and Purification

At harvest, the pH of the fermentation beer was adjusted to 6.0, the beer was filtered, and the filtrate (190 liters) was extracted with three 70-liter portions of ethyl acetate. Concentration of the extract *in vacuo* gave 400 g of a syrup. A 100-g portion of the concentrate was processed by counter-current distribution in a methanol - water - hexane system (3 : 1 : 4 by volume), employing six 500-ml separatory funnels with 200 ml each of upper and lower phases per funnel. A total of 12 transfers was made, moving the lower phase. Antibiotic activity was found in the first four fractions of the mobile phase, whereas the oily impurities remained in the upper phase of the first two funnels.

The active fractions were combined and concentrated to remove the organic solvents. Extraction of the resulting mixture with ethyl acetate, followed by concentration of the extract, gave 30 g of dry residue. A 10-g portion of this material was then dissolved in 20 ml of methanol and placed on a DEAE-cellulose column, packed with 40 g of Cellex-D (Bio-Rad Laboratories, Richmond, California) in methanol. The column was developed with methanol and 20-ml fractions were collected. The active fractions were combined and concentrated to dryness, yielding 1 g of powder. At this point, the bulk of the impurities had been removed from the active material. Further purification was effected by preparative thin-layer chromatography on silica gel plates (1,000-μ layer), using 10 % methanol in benzene. After development, the active material, appearing as a yellow band, was removed from the plate and eluted with acetone. Chromatography was usually repeated before the compound would crystallize readily. The yield was *ca.* 70 mg. Recrystallization from a benzene-methanol mixture gave yellow needles.

Characterization

Crystalline rabelomycin melts with decomposition at 193°C in an evaculated capillary. It is soluble in ethanol, acetone, and chloroform, and insoluble in water and petroleum ether. The following instrumentation was used for characterization: Perkin-

Fig. 1. Infrared spectrum of rabelomycin (KBr).

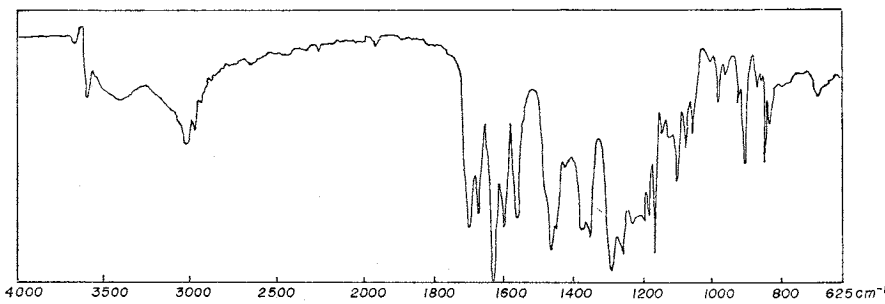
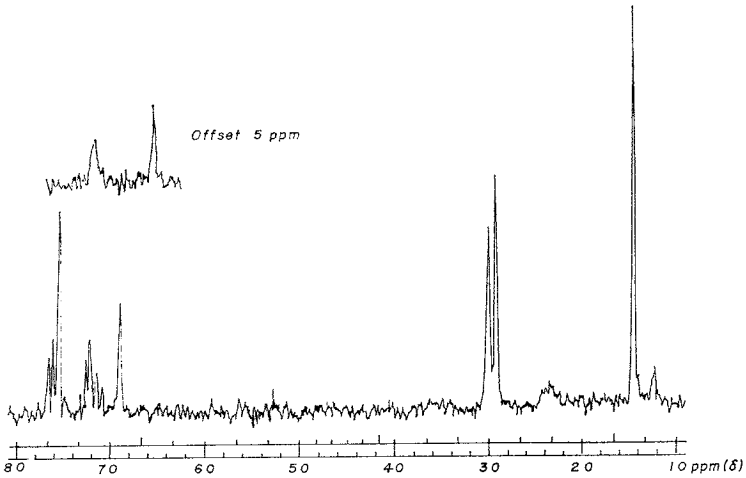


Fig. 2. NMR spectrum of rabelomycin.



Elmer Model 257 infrared spectrophotometer, Perkin-Elmer Model 402 ultraviolet spectrophotometer, Perkin-Elmer Model 141 polarimeter, Varian T-60 nmr spectrometer, and AEI Model MS-9 mass spectrometer. The infrared spectrum ($CHCl_3$) is shown in Fig. 1. The nmr spectrum (Fig. 2) was measured in $CDCl_3$ using tetramethylsilane as an internal standard: δ 1.47 (s, 3), 2.37 (broad, 1), 2.98 (s, 2), 3.06 (s, 2), 6.92 (s, 1), 7~8 (m, 3), 11.60 (s, 1), and 12.21 ppm (s, 1); uv max (neutral and acidic methanol): 228 nm (ε 26,600), 267 (28,800), 433 (8,000); uv max (basic methanol) 258 nm (ε 26,200); sh 282 (13,100), 325 (8,900), 507 (7,500); $[\alpha]_D$ $-102\pm10°$ (c 1.0 in $CHCl_3$). The high-resolution mass spectrum showed that the composition of the molecular ion (m/e 338) is $C_{19}H_{14}O_6$.

Anal. Calcd. for $C_{19}H_{14}O_6$: C 67.45, H 4.17.
Found: C 67.17, H 4.48.

Biological Properties

Rabelomycin is active against gram-positive microorganisms; its *in vitro* antibacterial spectrum is shown in Table 1. These results are similar to those reported for tetrangomycin[2].

Table 1. *In vitro* antibacterial activity of rabelomycin

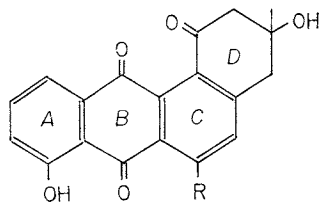
Microorganism	M.I.C. (μg/ml)
Staphylococcus aureus FDA 209P	6.3
Streptococcus pyogenes C203	1.2
Bacillus subtilis ATCC 6633	4.7
Escherichia coli ATCC 10536	>50.0
Salmonella schottmuelleri SC 3850*	>50.0
Pseudomonas aeruginosa SC 3840*	>50.0
Candida albicans CBS 35H	>50.0
Trichophyton mentagrophytes SC 2637*	>50.0

* Squibb culture collection

Discussion

The characterization data for rabelomycin indicate that it is a close relative of tetrangomycin, II, an unusual type of antibiotic recently reported[2,3]. Indeed, the nmr spectra of the two compounds only differ significantly in that the spectrum of rabelomycin has one additional chelated hydroxyl peak (11.60 ppm) and absorption in the aromatic region for one less proton. The multiplet in the 7 to 8 ppm region is consistent with a three-proton system expected for ring A, while the singlet at 6.92 ppm indicates an isolated proton on ring C. The empirical formula of rabelomycin differs from that of tetrangomycin by the addition of one oxygen atom. Attachment of a hydroxyl group on

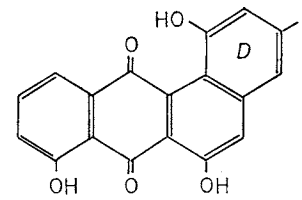
ring C, adjacent to the carbonyl group, gives a structure, I, (3, 4-dihydro-3, 6, 8-trihydroxy-3-methylbenz[*a*]anthracene-1,7,12(2H)-trione) that is consistent with the differences between the nmr spectra for rabelomycin and tetrangomycin. The interpretation of the rest of the spectrum is the same as that given for the nmr spectrum of tetrangomycin[3].



I R=OH
II R=H

The nmr spectrum alone does not establish I unambiguously as the structure for rabelomycin, but structural relationships provided by other spectroscopic methods eliminate alternative structures. The carbonyl absorption in the infrared spectrum (Fig. 1) shows that only one of the quinone carbonyls is chelated[4]. Also, the absorption at 1700 cm^{-1}, assigned to the carbonyl group on ring D, is at a somewhat unusual frequency due to steric and electronic interactions with the quinone carbonyl group, as had been observed for tetrangomycin[3]. While splitting between the proton on ring C and the neighboring methylene group of ring D is not observed directly, coupling can be demonstrated by double resonance: irradiation of the absorption at 3.06 ppm gives a pronounced sharpening of the 6.92 ppm resonance. The long-wavelength uv absorption maximum (433 nm) is, as expected, about the same as that for 1,8-dihydroxyanthraquinone (432 nm)[5]. The proposed location of the oxygen function on ring C is consistent with biogenesis from a hypothetical poly-β-ketomethylene precursor.

Rabelomycin is readily dehydrated to 1,6,8-trihydroxy-3-methylbenz[*a*]anthracene-7,12-dione, III, either thermally or by treatment with acid. To prepare III, a sample of rabelomycin was dissolved in concentrated sulfuric acid, giving a blood-red solution. Water was added with stirring, followed by extraction of III from the mixture with ethyl acetate. Purification by chromatography on silica gel, eluting with ethyl acetate - chloroform (1 : 4), and recrystallization from ethyl acetate gave a dark green solid: m.p. 204.5~205.0°C (evacuated capillary); ultraviolet max (neutral and acidic methanol): 234 nm (ε 39,9000), 268 (13,400), 325 (14,000), 455 (7,500), sh 550 (2,050); uv max (basic methanol): 235 nm (ε 38,800), 309 (15,200), 555 (8,300); infrared (KBr) 1632, 1607 cm^{-1}; nmr [$CF_3CO_2H - H_2SO_4$ (10 : 1)] δ *ca.* 1.2 (broad, 3), 2.54 (s, 3), 4.40 (s, 2), 7.05 (s, 1), 7.2~8.5 ppm (m, 4). (The solvent peak was used as an internal standard and was taken to be 11.30 ppm downfield from tetramethylsilane.) The high-resolution mass spectrum showed that the molecular ion (m/e 320) was $C_{19}H_{12}O_5$.



III

Anal. Cald. for $C_{19}H_{12}C_5$: C 71.24, H 3.78.
Found: C 71.34, H 4.08.

Rabelomycin is rather stable in glacial acetic acid, but was partially converted to III when a dilute solution in benzene was shaken with concentrated HCl for 3 minutes. In samples of rabelomycin that had been melted, III could easily be detected by thin-layer chromatography. However, unlike tetrangomycin[3], rabelomycin is not dehydrated by dilute base, perhaps because of the concentration of hydrogen bonds that would make rabelomycin more acidic. As a phenolate anion, it would be less subject to attack by base on ring D. The infrared spectrum of III shows that both carbonyl groups are chelated, supporting the structural relationship in rabelomycin between the carbonyl group of ring D and the carbonyl group that is not intramolecularly hydrogen bonded.

The nmr data for III in trifluoroacetic acid - concentrated sulfuric acid (10 : 1) (used for solubility reasons) indicate that III is protonated on ring D by the solvent. Similar behaviour has been observed for other aromatic systems in strong acid[6]. The broad peak at 1.2 ppm may be due to impurities. Phenolic protons are generally not observed in acid solvents[7] and we failed to observe the phenolic protons of phloroglucinol in this particular

solvent system. In nmr spectra of III taken in methylene chloride and in tetrahydrofuran, the phenolic protons absorb at about 12.0, 11.7 and 10.3 ppm. The latter peak is probably due to the ring D hydroxyl proton, which is chelated as expected[8].

The structure of another antibiotic, aquayamycin, having the unusual benzanthraquinone system has recently been reported[9].

Acknowledgements

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