ε_1-PYRROMYCINONE, A NEW ANTHRACYCLINONE FROM *Streptomyces galilaeus* JA 3043

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The formula *IIb* is proposed for ε_1-pyrromycinone, the anthracyclinone isolated from *Streptomyces galilaeus* JA 3043, on the basis of its UV, visible, IR, PMR and mass spectra.

Several anthracyclinones and two antibiotic substances have been isolated from the methanolic extract of the mycelium of the strain *Streptomyces galilaeus* JA 3043 (ref.[1,2]). The antibiotics named galirubine A and B, were identified as glycosides of the anthracyclinones aklavinone (*I*) and ε-pyrromycinone (*IIa*). We used the above mentioned mixture of glycosides as a source of ε-pyrromycinone in our experiments dealing with microorganisms. During the isolation we have found that ε-pyrromycinone is accompanied with a red substance, melting at 185–187°C, which changes its colour to violet in the alkaline medium similarly to ε-pyrromycinone.

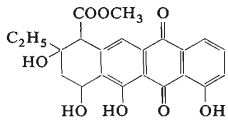
Infrared spectrum of this compound exhibits intense bands at 1601 cm^{-1} (chelated quinone C=O), 1730 cm^{-1} (ester C=O), 3500 and 3595 cm^{-1} (OH). The UV and visible spectra are undistinguishable from those of ε-pyrromycinone. Since ε-pyrromycinone and 1,4,5-trihydroxyanthraquinone have identical visible spectra and it is known that any change either in number or in the position of the hydroxyl groups changes the overall shape of the spectrum[3,4], the presence of an anthraquinone nucleus peri-substituted by three hydroxyl groups in the molecule is assumed. The molecular ion in the mass spectrum appears at *m/e* 414 and according to its elemental composition ($C_{21}H_{18}O_9$) contains one CH_2 group less than ε-pyrromycinone. The base peak *m/e* 378 ($C_{21}H_{14}O_7$) is formed by elimination of two molecules of water from this ion. Further intense fragments *m/e* 363, 347, 346 and 319 arise from the *m/e* 378 ion by loss of CH_3, CH_3O, CH_3OH and $COOCH_3$ (Scheme 1). The fragmentation pattern was confirmed by the registration of the metastable transitions by the method of Direct Analysis of Daughter Ions (DADI). In the PMR spectrum the signals of the protons analogous to those of the protons of ε-pyrromycinone, except the signal of the ethyl group which is replaced by the signal of the methyl group,

TABLE I
Comparison of PMR Spectra

ε-Pyrromycinone	ε_1-Pyrromycinone
1·07 t, $J = 6$ Hz, 3 H, CH_3CH_2	1·41 s, 3 H, CH_3
1·59 q, $J = 6$ Hz, 2 H, CH_3CH_2	—
3·69 s, 3 H, $COOCH_3$	3·61 s, 3 H, $COOCH_3$
2·18 d, $J = 14$ Hz, 1 H	2·13 d, $J = 15$ Hz, 1 H
2·44 dd, $J = 14$ and 3 Hz, 1 H	2·56 dd, $J = 15$ and 4 Hz, 1 H
4·09 s, 1 H	3·97 s, 1 H
5·19 mt, 1 H, OCH	5·26 mt, 1 H, OCH
7·12 s, 2 H, aromatic	7·29 s, 2 H, aromatic
7·51 s, 1 H, aromatic	7·68 s, 1 H, aromatic

s singlet, d doublet, t triplet, q quartet, dd doublet of doublets, mt multiplet.

can be found (Table I). A two-proton singlet at 7·21 p.p.m. was assigned to the two magnetically equivalent protons $H_{(2)}$ and $H_{(3)}$. Therefore the singlet of the remaining aromatic proton at 7·68 p.p.m. must be ascribed to a proton on another aromatic ring. Respecting the above demonstrated presence of the trihydroxyanthraquinone chromophor in the molecule, this proton can be situated either in the position 6 or 11. However, the chemical shift of the mentioned proton is closer to the value for $H_{(11)}$ in ε-pyrromycinone (7·51 p.p.m.) than to the value for $H_{(6)}$ in its isomer δ-rhodomycinone (*III*, 8·15 p.p.m.)[5]. The absence of an intense bisanhydrolactone fragment, typical for the mass spectra of anthracyclinones having a *peri*- relationship of the



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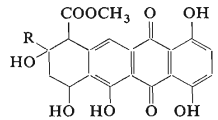
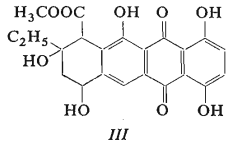
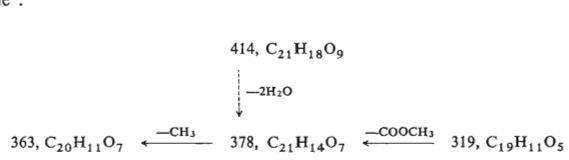


IIa, $R = C_2H_5$ *IIb*, $R = CH_3$



III

hydroxyl and carbomethoxyl groups[6,7], confirms the location of the hydroxyl group in the position 6. The situation of phenolic hydroxyls at $C_{(2)}$ and $C_{(3)}$, which would be also compatible with the observed PMR spectrum, can be excluded on the basis of visible spectrum which looks quite different in the case of 2,3-dihydroxyanthraquinone[8].



SCHEME 1

Map of Metastable Decompositions
— — — Accelerating voltage scan (defocusing method)
——— Electrostatic voltage scan (DADI, Direct analysis of daughter ions)

On the basis of the above summarized facts, we propose the structure *IIb* and the name ε_1-pyrromycinone (derived in the same manner as the name η_1-pyrromycinone for the lower homologue of η-pyrromycinone[9]) for our compound. Considering the isolation procedure we suppose that this compound is present in the streptomyces as a glycoside. From the viewpoint of biogenesis it is interesting that the same microorganism produces anthracyclinones containing $C_{(9)}$ ethyl group derived from propionic acid[8] as well as $C_{(9)}$ methyl group probably formed from the acetic acid.

EXPERIMENTAL

The melting point was measured with a Koffler hot-stage apparatus and was not corrected. The UV and visible spectra were measured on a Perkin-Elmer PE-402 spectrometer. IR spectra were recorded on a Zeiss UR-10 spectrometer in chloroform solution. Mass spectra were measured on a Varian MAT-311 spectrometer at 70 eV, ionizing current 1000 μA, ion source temperature 200°C; the sample was introduced *via* direct inlet system heated to 150°C. The elemental composition of the ions was determined using the peak-matching technique and the perfluoro kerosene standard with accuracy better than 2 p.p.m. A Tesla BS 487 (80 MHz) spectrometer was employed for recording the PMR spectra. The samples were measured in deuteriochloroform–hexadeuteriodimethyl sulphoxide solutions containing hexamethyldisiloxane as an internal standard.

Isolation of ε_1-Pyrromycinone

The strain *S. galilaeus* JA 3043 was cultivated in a 300 l tank under the described conditions[1]. The mycelium was separated, extracted with methanol and afterwards with methanol containing 0·5% of hydrochloric acid. The neutral extract was not further examined. The acid extract was

neutralized with the aqueous solution of sodium hydroxide and concentrated in vacuo to an aqueous residue, which was extracted with petroleum ether, diluted with an equal volume of methanol and extracted with chloroform. The last extract was filtered and the solvents were removed by distillation. The crude product (3·4 g) was portionwise chromatographed on a Sephadex LH-20 column (developed with methanol). The glycosidic fraction was indentified by comparison with authentic samples of galirubines using thin-layer chromatography (Silufol; methanol–formamide 10 : 1). The mixture of glycosides (1·4 g) was hydrolysed by 30 min heating with 0·1M-H_2SO_4 on a steam bath. After cooling, the reaction mixture was extracted with chloroform, the extract dried by filtration through anhydrous sodium sulphate and the solvent was removed. The residue (1·2 g) was chromatographed on silica gel impregnated with sodium hydrogen carbonate[10] (100 g, eluted with chloroform). The chromatography afforded 180 mg of ε-pyrromycinone and 16·8 mg of ε_1-pyrromycinone.

ε_1-pyrromycinone: m.p. 185—187°C (ethanol); $[\alpha]_D^{20}$ +117° (chloroform); $\lambda_{max}^{cyclohexane}$ 258, 289, 455 sh, 465 sh, 481, 492, 502 sh, 515 and 528 nm; mass spectrum *m/e* 414 ($C_{21}H_{18}O_9$, 3·2%, M^+), 396 ($C_{21}H_{16}O_8$, 7·5%, M — H_2O), 378 ($C_{21}H_{14}O_7$, 100%, M — 2 H_2O, a), 363 ($C_{20}H_{11}O_7$, 22·6%, a — CH_3), 347 ($C_{20}H_{11}O_6$, 32·3%, a — CH_3O), 346 ($C_{20}H_{10}O_6$, 11·8%, a — CH_3OH), 319 ($C_{19}H_{11}O_5$, 51·1%, a — $COOCH_3$), 291 ($C_{18}H_{11}O_4$, 17·2%, 319 — CO).

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