ISOLATION AND STRUCTURE OF A NEW ε-RHODOMYCIN COMPOUND PRODUCED BY A *STREPTOMYCES* SPECIES HPL Y-11472

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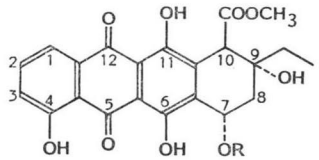
(Received for publication April 11, 1985)

In screening for novel antitumor compounds a *Streptomyces* species HPL Y-11472 was isolated and found to produce a new ε-rhodomycin compound along with ε-rhodomycinone[1,2,3]. This note describes the isolation and structure elucidation of this new compound.

Streptomyces species, HPL Y-11472, was fermented in 260 liters of medium containing (g per liter of H_2O) starch (10), glucose (10), malt extract (7.5), peptone (7.5), NaCl (3), $MgSO_4 \cdot 7H_2O$ (1), KH_2PO_4 (2), $CuSO_4 \cdot 5H_2O$ (0.007), $FeSO_4 \cdot 7H_2O$ (0.001), $MnSO_4 \cdot 4H_2O$ (0.008) and $ZnSO_4 \cdot 7H_2O$ (0.002) in a 390-liter fermenter at 26°C (±1°C) for 72 hours. The culture filtrate (about 230 liters) and mycelium (12 kg) were extracted with EtOAc and acetone respectively and the concentrated extracts were combined and dissolved in toluene. The toluene layer was washed with 0.2 M acetate buffer (pH 3.5) to remove basic components. One-tenth of the residue (2.8 g), obtained after removal of the toluene, was chromatographed on a silica gel column (eluted with 3% MeOH in $CHCl_3$) and further purified by the use of preparative TLC (developed with $CHCl_3$ - MeOH, 20: 1). Two compounds **1** and **2**, were isolated as orange red crystals.

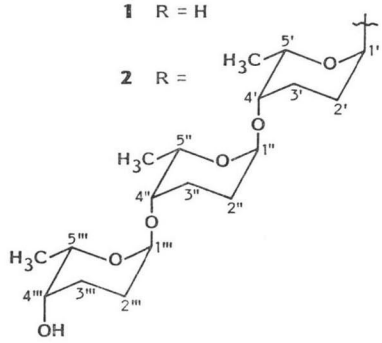
Compound **1**, mp 220°C (dec) analyzed for $C_{22}H_{20}O_9$ which was confirmed by mass spectroscopy *m/z* 428 (M^+). Its spectral data are as follows: IR ν_{max}^{KBr} cm^{-1} 3546, 1730, 1613, 1460, 1282; UV λ_{max}^{MeOH} 212, 234, 254 (sh), 296, 488, 510, 520, 546 and 558; $\lambda_{max}^{NaOH-MeOH}$ 580 and 616 nm; ^1H NMR $\delta_{TMS}^{CDCl_3}$ 1.15 (3H, t, J=8 Hz, CH_3), 2.28 (2H, br, CH_2), 3.71 (3H, s, $COOCH_3$), 4.28 (1H, s, 10-H), 5.35 (1H, br, 7-H), 7.34 (1H, dd, J=1.5 and 8 Hz, 3-H), 7.71 (1H, t, J=7 Hz, 2-H), 7.88 (1H, dd, J=1.5 and 8 Hz, 1-H), 12.11, 12.92 and 13.45 (3H, 3 × s, 3 × OH group). The ^1H NMR spectrum showed the presence of three aromatic protons whose chemical shifts and coupling pattern revealed that they are located adjacent to each other. Apart from this, the ^1H NMR showed a carboxymethyl group at δ 3.71 (the presence of a peak in the MS at *m/z* 369 which is $M^+ - COOCH_3$, corroborates this statement), three hydrogen bonded phenolic OH groups and one primary methyl group at δ 1.15. The presence of two non-phenolic OH groups was interpreted from the observation of MS fragmentation peaks at *m/z* 410 ($M^+ - H_2O$), and *m/z* 392 ($M^+ - 2H_2O$). In order to confirm the number and nature of the OH groups the compounds was acetylated with Ac_2O - pyridine at room temperature. The ^1H NMR of this acetylated derivative showed, apart from other signals, a singlet at δ 2.06 characteristic of an acetyl group on an aliphatic hydroxyl and three singlets at δ 2.44, 2.48 and 2.54 characteristic of phenolic acetyl groups. The OH group which did not acylate could be either a hindered or a tertiary one. The UV-visible spectrum of the compound is typical of anthracycline compounds. All these data are in agreement with those of ε-rhodomycinone[1,2,3].

Compound **2**: mp 130~135°C; analyzed for $C_{40}H_{50}O_{15}$. (*Anal* Calcd for $C_{40}H_{50}O_{15}$: C 62.3, H 6.5. Found: C 61.8, H 6.8); IR $\nu_{max}^{CHCl_3}$ cm^{-1}



1 R = H

2 R =



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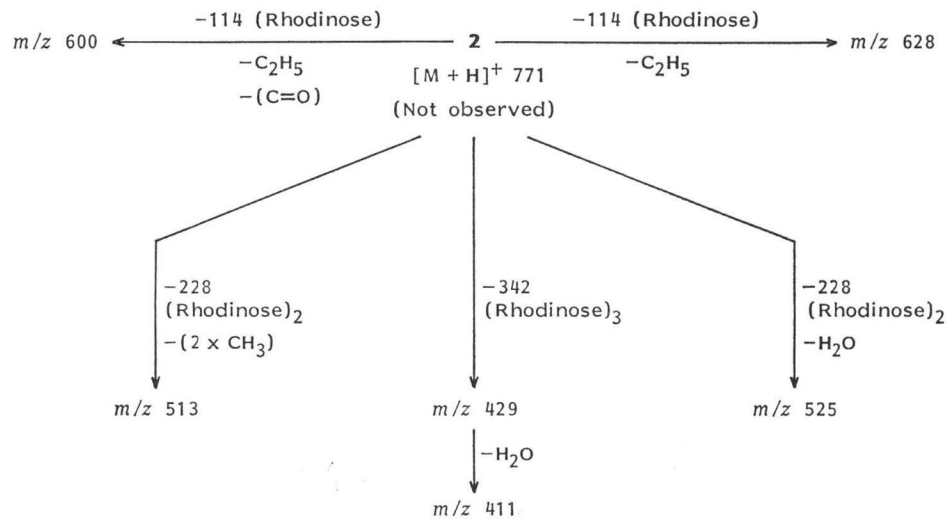
3472, 1710, 1587, 1567, 1429, 1277, 1193, 990, 962; UV λ_{max}^{MeOH} 235, 253 (sh), 294, 490, 510, 522, 544, 588; $\lambda_{max}^{NaOH-MeOH}$ 572 and 612 nm; ^{1}H NMR $\delta_{TMS}^{CDCl_3}$ 1.1 to 2.2 (12H, m, 4×CH_3), 1.6 (1H, br, OH), 1.7 to 2.25 (14H, m, 7×CH_2), 2.36 (2H, m, CH_2), 3.51 to 3.6 (3H, m, 4′-, 4″-, 4‴-H), 3.7 (3H, s, $COOCH_3$), 3.94 to 4.12 (3H, m, 5′-, 5″-, 5‴-H), 4.3 (1H, s, 10-H), 4.66 (1H, s, OH), 4.83 to 4.86 (2H, m, 1″-, 1‴-H), 5.28 (1H, br, 7-H), 5.45 (1H, br, 1′-H), 7.34 (1H, d, J=8 Hz, 3-H), 7.7 (1H, t, J=8 Hz, 2-H), 7.88 (1H, d, J=8 Hz, 1-H), 12.19, 12.88 and 13.52 (3H, 3×s, 3×OH group). These spectral data suggest that compound **2** is an anthracycline glycoside. On hydrolysis with 1 N HCl at 110°C for 2 hours it yielded an aglycone which was identical with compound **1** in all respects (mp, TLC, IR and ^{1}H NMR). The hydrolysate after removal of the aglycone by extraction into $CHCl_3$ was neutralized with Indion HIP (OH^-) resin (anion exchange resin manufactured by Ion Exchange (India) Ltd.) and freeze dried. This sugar portion on TLC using silica gel plates (60 F_{254}, E. Merck) in BuOH - AcOH - H_2O (4: 1: 1) showed a single spot with Rf value 0.73 (visualization was carried out with *p*-anisaldehyde spray reagent) which agrees with that reported for a rhodinose sugar[4] moiety. Further, the observation that compound **2** has more than one rhodinose unit was derived from both ^{1}H NMR data and partial hydrolysis experiments. Partial hydrolysis of compound **2** with 0.4 N HCl at 40°C for 4 hours gave a compound with Rf of 0.4 (in 5% MeOH in $CHCl_3$ on silica gel plate) which was in between ε-rhodomycinone (Rf 0.47) and compound **2** (Rf 0.35) along with free rhodinose sugar. This intermediate compound on further hydrolysis gave ε-rhodomycinone. Finally the ^{1}H NMR spectrum can be fully accounted for only on the basis of three rhodinose units. The attachment

Table 1. MIC of compound **2**.

Microorganism	MIC (μg/ml)
Staphylococcus aureus 209 P	125
S. aureus 20240	125
S. aureus R 85	125
S. aureus R 85/M	125
Bacillus subtilis	63
B. cereus ATCC 9634	63
Enterococcus faecalis ATCC 8043	125
Micrococcus luteus	<2
Candida albicans	>500
Escherichia coli 9632	>500
Proteus vulgaris	>500
P. stuartii	>500
Pseudomonas aeruginosa 20601	>500
Enterobacter cloacae	>500
Klebsiella pneumoniae	>500
Serratia marcescens	>500
Paracolon *Providencia*	>500
Citrobacter freundii	>500
Alcaligenes faecalis	250
Salmonella typhimurium	>500

Chart 1. FD mass spectral fragmentation pattern of compound **2**.



of the three rhodinose units to ε-rhodomycinone at 7-OH and not at 9-OH may be argued from the fact that the 9-OH is hindered and so far there are no reports in the literature of any anthracycline glycosides with the sugars attached at 9-OH. The field desorption mass spectrum of compound **2** did not give a M^+ peak, but the fragmentation pattern depicted in Chart 1 offers additional evidence for the structure to be ε-rhodomycinone-7-(rhodinose)$_3$. The minimum inhibitory concentrations (MIC) of compound **2** against various microorganisms are given in Table 1.

Acknowledgments

We thank Mr. H. B. CHAUBAL for his technical assistance and Mr. K. R. DESIKAN for excellent fermentation support.

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