NEW RHODOMYCINONE FROM THE STRAIN *Streptomyces griseoruber* 4620

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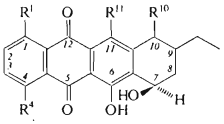
New anthracyclinone, 10-deoxy-β-rhodomycinone (*V*), was isolated from the strain *Streptomyces griseoruber* 4620 besides β-rhodomycinone (*I*), β-isorhodomycinone (*II*), α_2-rhodomycinone (*III*), and ε-rhodomycinone (*IV*). The identification and the structure elucidation are based on the UV, CD, IR, ^{1}H-NMR and mass spectra.

The antibiotics beromycin A, B and C have been isolated from the strain *Streptomyces griseoruber* 4620 and preliminarily reported as the members of the anthracycline group[1]. Working with one variant of this strain, we now proved the presence of another antibiotics in the extracts of the fermentation broth and the mycelium[2]. The hydrolysis of the mixture of these antibiotics afforded four already known aglycones: β-rhodomycinone[3] (*I*), β-isorhodomycinone[4] (*II*), α_2-rhodomycinone[5] (*III*) and ε-rhodomycinone[6] (*IV*). The mixture contains another red compound melting at 218 – 210°C. The subject of this communication is the structural proof of the isolated aglycones.

The unknown compound has a summary formula $C_{20}H_{18}O_7$ (high resolution mass spectrometry). Its ^{1}H-NMR spectrum contains a three-proton triplet of the primary methyl group at 1·08 ppm (J = 7·3 Hz) and a corresponding two-proton quartet at 1·92 ppm (J = 7·3 Hz), a two-proton singlet at 1·82 ppm, a doublet of doublets at 3·13 ppm (J = 15·9 and 1·8 Hz), a doublet at 4·33 ppm (J = 4·9 Hz), a multiplet at 4·76 ppm (J = 4·9, 1·8 and 1·2 Hz), a broad singlet at 5·09 ppm, a doublet of doublets at 7·31 ppm (J = 7·2 and 2·2 Hz), a triplet at 7·70 ppm (J = 7·2 Hz), a doublet of doublets at 7·88 ppm (J = 7·2 and 2·2 Hz) and three singlets at 12·27, 12·80 and 13·88 ppm. The last three protons and the protons giving rise to a doublet at 4·33 ppm and to a singlet at 5·09 ppm are exchanged for deuterium upon the addition of the perdeuterioacetic acid. That means that the molecule contains one secondary and four tertiary hydroxyl groups. Using the chemical shift information, three of them can be assigned to phenolic hydroxyls. Two of the total

three aromatic protons exhibit each one *ortho*- and one *meta*-coupling; the remaining aromatic proton has two *ortho*-couplings. Therefore, these protons represent a system of three vicinal aromatic protons in the same ring. The chemical shift of the methine proton due to the secondary alcohol group suggests its location in the α-position to a double bond. Its small couplings to the protons at 2·75 and 3·13 ppm, whose mutual coupling ($J = J_{gem} = 15{\cdot}9$ Hz) allows their unambiguous assignment to the methylene group protons and indicates its quasiequatorial orientation[7]. The absence of any further couplings of the above mentioned methylene protons implies that the carbon atom vicinal to this does not carry any protons. The ethyl group must be attached to a similar atom since it exhibits the spectrum of the A_3B_2 type. Also the two-proton singlet at 1·82 ppm can be interpreted as the signal of the protons of an isolated methylene group. The infrared spectrum contains a band at 1600 cm^{-1} (chelated quinone carbonyl). This fact plus the information on the number of the phenolic groups indicates the presence of the 1,4,5-trihydroxyanthraquinone system.



There is a missing OH group at $C_{(9)}$

	R^1	R^4	R^{10}	R^{11}
I,	H	OH	OH	OH
II,	OH	OH	OH	OH
III,	OH	OH	OH	H
IV,	H	OH	$COOCH_3$	OH
V,	H	OH	H	OH
VI,	OH	H	H	OH

This deduction is supported by the similarity of the electronic spectrum of this compound with that of β-rhodomycinone (*I*) indicating the same substitution pattern of the chromophore by the hydroxyl groups[8,9]. However, according to the elemental composition, our compound contains one oxygen atom less. The observed fragmentation, especially the elimination of two molecules of water and the loss of C_4H_8O are quite common with C_{20}-anthracyclinones. The elimination of C_4H_8O from the molecular ion is interpreted as a retro-Diels–Alder rearrangement[10] and can be used as an evidence for the positioning the ethyl group in the β-position to the aromatic system. The loss of C_3H_5O corresponds to the splitting of $C_{(9)}$ with both its substituents accompanied by the hydrogen transfer; the same fragmentation was described by Brockmann and coworkers[11] with 10-deoxy-γ-rhodomycinone. All the observed facts are satisfied by the structures *V* and *VI*. We prefer the former as the

more probable one from the viewpoint of biogenesis. The absolute configuration at $C_{(7)}$ was determined as 7S from the circular dichroism (CD) curve since 10-deoxy--β-rhodomycinone (*V*) has the same Cotton effect as the anthracyclinones measured by Brockmann and coworkers[12,13] in the region 270–380 nm.

EXPERIMENTAL

The melting point was determined in a Kofler hot stage. Electronic spectra were measured on a Cary 118 C spectrophotometer in cyclohexane or chloroform. The infrared spectra were measured in KBr pellets on a Unicam SP 200 instrument. Mass spectra were measured on a Varian MAT 311 spectrometer at the energy and current of ionizing electrons 11 aJ (70 eV) and 1 mA, respectively; the ion source temperature was 200°C, the temperature of direct inlet system was 150–170°C. High resolution measurement of all ions discussed above was made by the "peak matching" technique with perfluorokerosene as the standard (error ± 5 ppm). The ^{1}H-NMR spectra were measured on a Jeol FX 60 spectrometer (FT mode, 59·797 MHz) at 25°C in deuteriochloroform with some hexadeuteriodimethyl sulfoxide added or in pentadeuteriopyridine. Tetramethylsilan was employed as an internal standard. Chemical shifts were calculated from the digitally obtained address differences (±0·02 ppm). The circular dichroism (CD) curve was measured on a Rousell–Jouan CD 185/II dichrograph at 25°C in a 0·1 cm cell in dioxane. The data are given in the molecular ellipticities $[\Theta]$ deg cm^2 dmol^{-1}. The R_F values were obtained from TLC on Silufol 20 in the system chloroform–methanol 14 : 1. The melting points of our isolates agreed with the literature data within 2°C. The positions of the UV maxima were identical within 2 nm. The maximum difference of the ^{1}H-NMR parameters was 0·37 ppm. The agreement of the mass spectra involved *m/z* values and relative intensities of the eight most abundant peaks.

Isolation of Rhodomycinones

The cultivation of the strain *S. griseoruber* 4620 in a 300 l tank, the work-up of the mycelium and the fermentation broth, the isolation of the mixture of antibiotics, their hydrolysis and the isolation of individual rhodomycinones is described elsewhere[2].

β-*Rhodomycinone* (I): m.p. 225–226°C (CH_3COOH); R_F 0·44. UV VIS spectrum λ_{max} (cyclohexane): 394, 464, 481, 492, 514, 528 nm. IR spectrum (KBr): 1 600 cm^{-1} (chelated quinone). Mass spectrum: *m/z* 386 ($C_{20}H_{18}O_8$, 2%, M$^+$), 368 ($C_{20}H_{16}O_7$, 12%, M–H_2O), 350 ($C_{20}H_{14}$·O_6, 42%, M–2 H_2O), 335 ($C_{20}H_{15}O_5$, 19%), 335 ($C_{19}H_{11}O_6$, 19%), 334 ($C_{20}H_{14}O_5$, 100%), 319 ($C_{19}H_{11}O_5$, 36%), 314 ($C_{16}H_{10}O_7$, 24%, M–C_4H_8O), 312 ($C_{17}H_{12}O_6$, 21%), 311 ($C_{17}H_{11}O_6$, 21%), 307 ($C_{18}H_{11}O_5$, 14%), 296 ($C_{16}H_8O_6$, 44%, M–C_4H_8O–H_2O), 294 ($C_{17}H_{10}O_5$, 19%) 286 ($C_{15}H_{10}O_6$, 10%, M–C_4H_8O–CO), 284 ($C_{16}H_{12}O_5$, 12%), 283 ($C_{16}H_{11}O_5$, 14%), 270 ($C_{15}H_{10}O_5$, 19%). ^{1}H-NMR spectrum (C_5D_5N): 1·35 (t, 7·3 Hz, 3 H), 2·17 (q, 7·3 Ht, 2 H), 2·53 (mt, 2 H), 5·53 (s, 1 H), 5·57 (mt, 1 H), 7·39–8·04 (mt, ABC, 3 H).

β-*Isorhodomycinone* (II): m.p. 215–217°C ($CHCl_3$); R_F 0·45. UV/VIS spectrum λ_{max} (chloroform): 492, 514, 524, 551, 563 nm. IR (KBr): 1 595 cm^{-1} (chelated quinone). Mass spectrum: *m/z* 402 ($C_{20}H_{18}O_9$, 6% M$^+$), 384 ($C_{20}H_{16}O_8$, 31%, M–H_2O), 368 ($C_{20}H_{16}O_7$, 26%), 366 ($C_{20}H_{14}O_7$, 100%, M–2 H_2O), 351 ($C_{19}H_{11}O_7$, 54%), 350 ($C_{20}H_{14}O_6$, 85%), 335 ($C_{19}H_{11}O_6$, 20%), 335 ($C_{20}H_{15}O_5$, 10%), 334 ($C_{20}H_{14}O_5$, 25%), 330 ($C_{16}H_{10}O_8$, 36%, M–C_4H_8O), 327 ($C_{17}H_{11}O_7$, 46%), 323 ($C_{18}H_{11}O_6$, 15%), 319 ($C_{19}H_{11}O_5$), 9%), 314 (C_{16}·$H_{10}O_7$, 32%), 312 ($C_{16}H_8O_7$, 26%, M–C_4H_8O–H_2O), 312 ($C_{17}H_{12}O_6$, 52%), 311 ($C_{17}H_{11}$·O_6, 48%), 310 ($C_{17}H_{10}O_6$, 45%), 296 ($C_{17}H_{12}O_5$, 31%), 286 ($C_{15}H_{10}O_6$, 30%), 284 ($C_{16}H_{12}$·

.O_5, 13%), 283 ($C_{11}H_{11}O_5$, 21%), 270 ($C_{15}H_{10}O_5$, 21%). ^{1}H-NMR spectrum (C_5D_5N): 1·37 (*t*, *J* = 7·3 Hz, 3 H), 2·20 (q, *J* = 7·3 Hz, 2 H), 2·51 (mt, 2 H), 5·49 (s, 1 H), 5·23 (mt, 1 H), 7·35 (s, 2 H).

α$_2$-Rhodomycinone (III): m.p. 207—209°C (CH_3COOH); R_F 0·38. UV/VIS spectrum λ_{max} (cyclohexane): 465, 483, 495, 517, 531 nm. IR spectrum (KBr): 1 595 cm^{-1} (chelated quinone). Mass spectrum: *m/z* 386 ($C_{20}H_{18}O_8$, 2%, M^+), 368 ($C_{20}H_{16}O_7$, 49%, $M-H_2O$), 352 ($C_{20}H_{16}$. .O_6, 16%), 350 ($C_{20}H_{14}O_6$, 14%, $M-2\,H_2O$), 335 ($C_{19}H_{11}O_6$, 16%), 334 ($C_{20}H_{14}O_5$, 34%), 319 ($C_{20}H_{11}O_5$, 19%), 314 ($C_{16}H_{10}O_7$, 34%, $M-C_4H_8O$), 312 ($C_{17}H_{12}O_6$, 61%), 311 (C_{17}. .$H_{11}O_6$, 100%), 307 ($C_{18}H_{11}O_5$, 8%), 296 ($C_{16}H_8O_6$, 60%, $M-C_4H_8O-H_2O$), 296 ($C_{17}H_{12}$. .O_5, 15%), 294 ($C_{17}H_{10}O_5$, 27%), 283 ($C_{16}H_{11}O_5$, 14%), 270 ($C_{15}H_{10}O_5$, 18%), 268 ($C_{15}H_{18}$. .O_5, 16%). ^{1}H-NMR spectrum (C_5D_5N): 1·33 (t, *J* = 7·3 Hz, 3 H), 2·23 (q, *J* = 7·3 Hz, 2 H), 2·40 (dd, *J* = 14·7 and 1·2 Hz, 1 H), 2·78 (dd, *J* = 14·7 and 4·9 Hz, 1 H), 5·25 (mt, 1 H), 5·53 (s, 1 H), 7·33 (s, 2 H), 8·35 (s, 1 H).

ε-*Rhodomycinone* (IV): m.p. 210—212°C (CH_3COOH); R_F 0·82. UV/VIS spectrum λ_{max} (cyclohexane): 483, 493, 515, 529 nm. IR spectrum (KBr): 1 610 cm^{-1} (chelated quinone), 1 740 cm^{-1} (ester C=O). Mass spectrum: *m/z* 428 ($C_{22}H_{20}O_9$, 11%, M^+), 410 ($C_{22}H_{18}O_8$, 3%, $M-H_2O$), 392 ($C_{22}H_{16}O_7$, 11%, $M-2\,H_2O$), 376 ($C_{21}H_{12}O_7$, 25%), 368 ($C_{20}H_{16}O_7$, 6%), 360 ($C_{21}H_{12}O_6$, 100%, $M-2\,H_2O-CH_3OH$), 351 ($C_{20}H_{15}O_6$, 9%), 350 ($C_{20}H_{14}O_6$, 6%), 349 ($C_{20}H_{13}O_6$, 10%), 339 ($C_{18}H_{11}O_7$, 13%), 335 ($C_{20}H_{15}O_5$, 10%), 334 ($C_{20}H_{14}O_5$, 9%), 333 ($C_{20}H_{13}O_5$, 10%) 323 ($C_{18}H_{11}O_6$, 14%), 322 ($C_{18}H_{10}O_6$, 13%), 321 ($C_{18}H_9O_6$, 7%), 319 ($C_{19}H_{11}O_5$, 3%), 294 ($C_{17}H_{10}O_5$, 9%), 203 ($C_{11}H_7O_4$, 5%), 202 ($C_{11}H_6O_4$, 5%), 121 ($C_7H_5O_2$, 7%), 57 (C_3H_5O, 25%). ^{1}H-NMR spectrum ($CDCl_3$): 1·14 (t, *J* = 7·3 Hz, 3 H), 2·05 (q, *J* = = 7·3 Hz, 2 H), 2·28 (mt, 2 H), 3·47 (br s, OH), 3·72 (s, 3 H), 4·27 (s, 1 H), 5·43 (mt, 1 H), 7·28 to 7·96 (mt, 3 H), 12·11 (s, 1 H), 12·92 (s, 1 H), 13·45 (s, 1 H).

10-*Deoxy*-β-*rhodomycinone* (V): red-orange needles m.p. 218—220°C (CH_3COOH); R_F 0·67. UV/VIS spectrum λ_{max} (cyclohexane): 391, 411, 464, 484, 495, 517, 531, 564 nm; λ_{max} (ε) (chloroform): 391 (2 990), 412 (3 950), 467 (13 330), 496 (17 740), 519 (13 100), 531 (12 600), 564 nm, (1750). IR spectrum (KBr): 1 600 cm^{-1} (chelated quinone). Mass spectrum: *m/z* 370 ($C_{20}H_{18}O_7$, 31%, M^+), 352 ($C_{20}H_{16}O_6$, 31%, $M-H_2O$), 336 ($C_{20}H_{16}O_5$, 8%), 334 ($C_{20}H_{14}$. .O_5, 15%, $M-2\,H_2O$), 323 ($C_{19}H_{15}O_5$, 15%), 311 ($C_{17}H_{11}O_6$. 7%), 307 ($C_{18}H_{11}O_5$, 10%), 298 ($C_{16}H_{10}O_6$, 37%, $M-C_4H_8O$), 296 ($C_{17}H_{12}O_5$, 25%), 295 ($C_{17}H_{11}O_5$, 51%, $M-C_3H_5O-$ $-H_2O$), 286 ($C_{15}H_{10}O_6$, 9%), 285 ($C_{15}H_9O_6$, 10%), 270 ($C_{15}H_{10}O_5$, 100%, $M-C_4H_8O-CO$), 255 ($C_{14}H_7O_5$, 11%). ^{1}H-NMR spectrum ($CDCl_3$ + $(CD_3)_2SO$): 1·08 (t, *J* = 7·3 Hz, 3 H), 1·82 (mt, 2 H), 1·92 (q, *J* = 7·3 Hz, 2 H), 2·75 and 3·13 (AB part of an ABXY system, J_{AB} = = 15·9 Hz, 2 H), 4·33 (d, *J* = 4·9 Hz, 1 H), 4·76 (mt, *J* = 4·9, 1·8 and 1·2 Hz, 1 H), 7·31 (dd, *J* = 7·2 and 2·2 Hz, 1 H), 7·70 (t, *J* = 7·2 Hz, 1 H), 7·88 (dd, *J* = 7·2 and 2·2 Hz, 1 H), 12·27 (s, 1 H), 12·80 (s, 1 H), 13·88 (s, 1 H). Circular dichroism (dioxan): 275 nm −1 740, 285 nm −1 450, 303 nm 0, 320 nm +2 620, 355 nm +3 780.

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