ANTHRAQUINONES RELATED TO ANTHRACYCLINONES FROM THE MUTANT STRAIN *Streptomyces galilaeus* J-14

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The anhydro derivative of ε-pyrromycinone *IX* and three anthraquinones *X*—*XII* related to anthracyclinones were found as minor metabolites of the mutant strain *Streptomyces galilaeus* J—14. Proposed structures are based on the spectroscopic measurements.

Streptomyces galilaeus is the producer of a mixture of glycosidic antibiotics belonging to the anthracycline group[1-7] the aglycones of which are mostly aklavinone (*I*) and ε-pyrromycinone (*II*). In addition to glycosides, 7-deoxy derivatives of the mentioned aglycones *III* and *IV* (ref.[1-5]), product of the reductive coupling of two molecules of *III* (ref.[5]), bisanhydro derivatives *V* and *VI* (ref.[1,5]), ε_1-pyrromycinone[8] (*VII*), two stereosiomers of aklavinone[9,10] and bisanhydro-ε-isorhodomycinone (ref.[11]) *VIII* have been isolated from this source. Using a multi-stage selection, the mutants with increased production of ε-pyrromycinone (*II*) glycosides and mutants blocked in the antibiotics production were obtained[12]. The mutant J—14 was isolated from the population after the treatment with nitrous acid. It has a blocked glycosidation step of the biosynthesis of anthracyclines, does not produce any glycosides and accumulates free aglycones,

Its major metabolite is ε-pyrromycinone (*II*). As the other components there were found aklavinone (*I*), ε_1-pyrromycinone (*VII*), 7-deoxy derivatives *III*, *IV* and bisanhydro derivatives *V* and *VI*. In addition to them four other compounds were chromatographically detected. This contribution is devoted to their isolation and identification.

Red compound, having chromatographic mobility between aklavinone (*I*) and 7-deoxypyrromycinone (*IV*), has a summary formula $C_{22}H_{18}O_8$. It contains one molecule of water less than *II* and has a very similar mass spectrum[13]. The base peak m/z 392 is formed by elimination of water from the molecular ion. The difference between ^{1}H-NMR spectrum of this compound and that of *II* (Table I) comprises missing signal of the H—10 proton and downfield shift of the methyl ester and ethyl methylene signals. These facts allow to place the double bond between the atoms $C_{(9)}$ and $C_{(10)}$, which leads to the formula *IX*. It is interesting that this

compounds have not yet been reported[14] among the products of the dehydration of *II*.

The mass spectrum of the orange compound (m.p. 176–178°C), less polar than 7-deoxyaklavinone (*III*)) indicates that this compound is its isomer. However, there is one carbonyl band (1 700 cm^{-1}) more in its infrared spectrum. The comparison of the mass and ^{1}H-NMR spectra (Table I), chromatographic behaviour and mixed melting point proved that this compound is identical to the recently described (ref.[15]) compound *X*. Next compound on the chromatogram (m.p. 177–179°C) is an isomer of 7-deoxypyrromycinone (*IV*). Its infrared spectrum also contains one carbonyl band (1 700 cm^{-1}) more than that of *IV*. The peaks in the mass spectrum

TABLE I

Comparison of the ^{1}H-NMR Spectra of the Investigated Compounds

Chemical shifts expressed in the δ-scale, coupling constants in Hz given in parentheses; s singlet, d doublet, t triplet, q quartet, mt multiplet.

Proton	*II*	*IX*	*III*[a]	*X*[a]	*XI*	*XII*
H–1	—	—	7·88 dd (7·8, 2·4)	7·85 dd (7·4, 1·0)	—	7·88 dd (7·8, 2·4)
H–2	7·28 s[b]	7·33 s[b]	7·70 t (7·8)	7·68 t (7·4)	7·70 s[b]	7·69 t (7·8)
H–3	7·28 s[b]	7·33 s[b]	7·30 dd (7·8, 2·4)	7·31 dd (7·4, 1·0)	7·30 s[b]	7·27 dd (7·8, 2·4)
H–7	5·25 t $\sum J = 7{\cdot}5$	5·35 q $\sum J = 7{\cdot}3$	2·20–3·10[c]	2·69–3·14[c]	2·70–3·04[c]	—
H–8	2·52 mt	2·78 mt	2·10–3·10[c]	2·69–3·14[c]	2·70–3·04[c]	—
H–10	3·90 s	—	3·95 s	3·90 s[b]	3·94 s[b]	3·80 s[b]
H–11	7·69 s	7·70 s	7·65 s	7·70 s	7·76 s	7·73 s
CH_3CH_2	1·03 t (7·3)	1·18 t (7·3)	1·08 t (7·3)	1·06 t (7·3)	1·08 t (7·2)	1·20 t (7·3)
CH_3CH_2	1·53 q (7·3)	2·47 q (7·3)	1·65 q (7·3)	2·45 q (7·3)	2·41 q (7·2)	3·03 q (7·3)
$COOCH_3$	3·52 s	3·96 s	3·73 s	3·72 s	3·74 s	3·70 s
OH[d]	12·00 s 12·70 s 12·85 s	12·25 s 12·66 s 12·96 s	12·09 s 12·48 s	12·05 s 12·52 s	12·27 s 12·66 s 13·05 s	11·93 s 12·45 s

[a] Ref.[15]; [b] 2 H; [c] AA'BB' system; [d] exchangeable protons.

(Fig. 1) are shifted of 16 mass units with respect to *X* and according to the high-resolution measurement contain each one oxygen atom more. The comparison of the both ^{1}H-NMR spectra (Table I) shows that this compound is a 1-hydroxyanalogue of *X*, formula *XI*. The formation of this compound by heating of *IV* was mentioned by Brockmann[14]. The compounds *X* and *XI* could be either the precursors of *III* and *IV* since the aldolization of *XI* leading to the racemic *IV* was described[14], or the products of their thermal decomposition.

The less polar compound among the four substances discussed above has an elemental composition $C_{20}H_{16}O_7$. Its infrared spectrum contains besides the bands of chelated and unchelated quinone carbonyls (1620 and 1660 cm^{-1}) and band of an ester carbonyl (1735 cm^{-1}) one carbobyl band more: 1685 cm^{-1} (conjugated carbonyl). The UV/VIS spectrum is similar to that of *X*. The ^{1}H-NMR spectra of this compound and that of *X* have also much common (Table I). However, the signals of the AA'BB' system of the protons on $C_{(7)}$ and $C_{(8)}$ are missing and the signal of the ethyl methylene is shifted downfield. The ^{13}C-NMR spectrum (Table II) contains signals of all 20 carbons. According to their off-resonance multiplicity, there are two methyl groups, two methylene groups and four sp^2-hybridized carbon methines in the molecule. The remaining carbon atoms are quaternary. In the low-field region of the ^{13}C-NMR spectrum resonate the ester carbonyl (170·2 ppm),

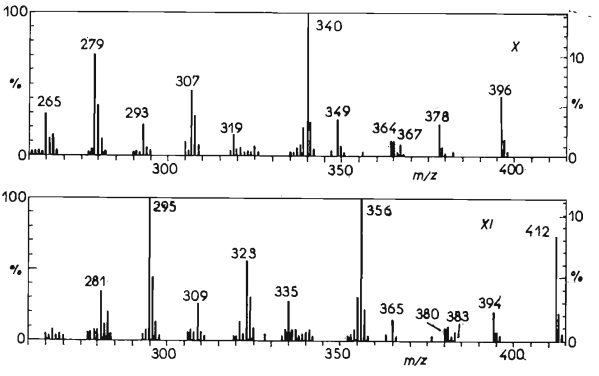


FIG. 1

Mass Spectra of the Compounds *X* and *XI*

Left coordinate: % of relative intensity, right coordinate: % of total ion current.

chelated and unchelated quinone carbonyls (192·1 and 181·0 ppm) and an another carbon at 198·6 ppm. Its chemical shift corresponds to a conjugated carbonyl involved in some hydrogen bonding. The comparison of ^{13}C-NMR spectra of the compounds *III*, *X* and *XII* see Table II shows that the compounds *X* and *XII* have common 1,8-dihydroxyanthraquinone skeleton. Its substituents are $—CH_2COOCH_3$ and $—COCH_2CH_3$. Since the side chain carbonyl is chelated, this chain must be attached in the vicinity of some hydroxyl. These requirements are not met with the ring A since it has three vicinal protons. Therefore, the ring C remains and the substituent under discussion must be attached to $C_{(7)}$ (anthraquinone numbering). This

TABLE II

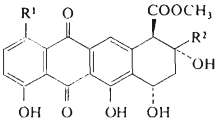
Comparison of the ^{13}C-NMR Data of Compounds *I*, *III*, *X* and *XII*

Chemical shifts expressed in the δ-scale. The multiplicity in the off-resonance spectra corresponds to the formulas.

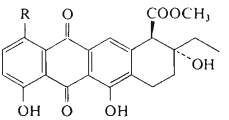
Carbon atom	*I*[a]	*I*[b]	*III*[b]	*X*[b]	*XII*
1	120·0	120·3	119·8	119·9	120·3
2	137·1	137·5	137·0	137·2	137·6
3	124·6	124·9	124·5	124·6	125·0
4	161·9	161·3	160·9	161·3	160·0
5	192·4	192·8	192·7	193·0	192·7
6	162·3	162·7	158·2	162·2	162·7
7	71·0	62·5	19·9	21·2	—
8	33·7	34·8	28·2	40·4	—
9	71·7	71·7	71·5	210·4	198·6
10	57·1	56·9	55·9	39·4	38·7
11	120·8	121·4	121·1	122·3	122·4
12	181·0	181·3	181·4	180·4	181·0
13	32·1	32·5	32·2	35·9	37·4
14	6·7	6·8	6·6	7·8	7·7
15	171·0	171·3	171·4	170·6	170·2
16	52·4	52·5	52·5	52·4	52·4
4a	114·4	114·7	114·2	114·4	114·5
5a	115·6	115·8	116·7	115·9	118·4
6a	132·7	132·9	126·9	137·0	137·0
10a	142·5	142·7	141·9	142·6	141·5
11a	131·2	132·7	130·7	131·1	130·9
12a	133·3	133·6	130·7	133·7	133·5

[a] Aglycone part of the glycoside aklavin, data from the ref.[22] preferred over that of ref.[21]; [b] ref.[15].

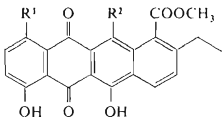
deduction is supported by the observed multiplicity of the $C_{(11)}$ signal in the undecoupled ^{13}C-NMR spectrum (doublet of triplet ^1J 165·0 Hz, ^3J 3·9 Hz) which is consistent with the relative *ortho*-position of the $-CH_2COOCH_3$ group to $C_{(11)}$ only. Thus, we obtain the formula *XII*. The fragmentation upon electron impact can be rationalized according to the Scheme 1. This compound, related to anthracyclinones, can be formed by the same biosynthetic pathway[16-20] from the oligoketide precursor one acetate unit shorter.



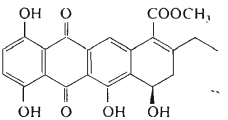
I, $R^1 = H$, $R^2 = CH_2CH_3$
II, $R^1 = OH$, $R^2 = CH_2CH_3$
VII, $R^1 = OH$, $R^2 = CH_3$



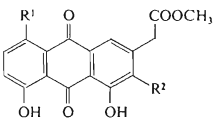
III, R = H
IV, R = OH



V, $R^1 = R^2 = H$
VI, $R^1 = OH$, $R^2 = H$
VIII, $R^1 = R^2 = OH$



IX



X, $R^1 = H$, $R^2 = CH_2CH_2COCH_2CH_3$
XI, $R^1 = OH$, $R^2 = CH_2CH_2COCH_2CH_3$
XII, $R^1 = H$, $R^2 = COCH_2CH_3$

Scheme 1
Mass Spectroscopic Fragmentation of the Compound *XII*

EXPERIMENTAL

The melting points were determined in the Kofler apparatus. Ultraviolet and visible spectra were measured in cyclohexane on a Cary 118 C spectrometer, infrared spectra were measured

in KBr pellets using the Unicam SP-200 instrument. The mass spectrometer used in this study was Varian MAT-311; ion source temperature was 200°C, direct inlet temperature was 150°C (130°C with *XII*), energy of ionizing electrons 11 aJ (70 eV), ionizing electron current 1 mA. The elemental composition of the ions was determined using the peak-matching technique ±5 ppm and perfluorokerosene as the standard. Metastable ions were detected from the field-free region between the magnetic and electrostatic sectors by electrostatic field scanning. ^{1}H- and ^{13}C-NMR spectra were measured on a Jeol FX-60 NMR spectrometer (59·797 and 15·036 MHz, FT mode) at 25°C in deuteriochloroform with tetramethylsilane as an internal standard. Chemical shifts were calculated with accuracy ±0·005 and ±0·06 ppm from the digitally obtained address differences. The assignments given in the Table II are based on the off-resonance determined signal multiplicity, noise off-resonance decoupling, selective decoupling, comparison of the related compounds and general chemical shift considerations.

Strain and Cultivation

The mutant strain *Streptomyces galileus* J—14 was isolated from the population subjected to the nitrous acid treatment (50 mM-$NaNO_2$, pH 4·0, 25 min). The strain was maintained on the yeast--maltose agar[23]. For the submerged cultivation a medium according to Bradler[24] with a modified glucose content (1% for inoculum, 5% for the second vegetative generation), was used. The fermentation flasks were inoculated with 5% of the 24 h old inoculum and cultivated 120 h on a reciprocal shaker (frequency 1·6 Hz, amplitude 80 mm) at 27°C.

Isolation

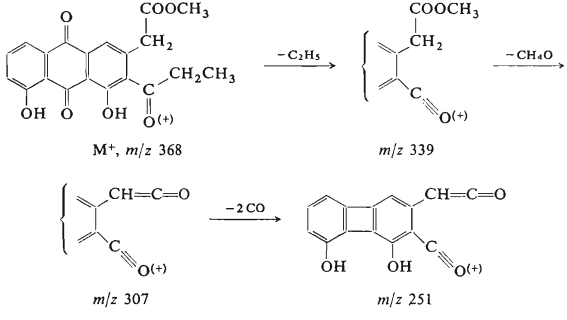
After 120 hours of fermentation, the mycelium from 180 flasks (10 l) was filtered off and extracted by methanol. The extract was evaporated *in vacuo* and the residue extracted by chloroform. This extract was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue (7·8 g) was chromatographed on a silica gel (desactivated by 17% of water) column; for the elution benzene and the mixture benzene–chloroform (50 : 1—3 : 2) were used. The mixture was resolved into eight fractions (volumes 200, 600, 1 400, 600, 800, 1 400, 1 200, and 1 000 ml). Fractions 3, 4, and 5 were further purified by TLC on the precoated silica gel plates Silufol (Kavalier, Czechoslovakia) by the solvent mixtures: heptane–chloroform–methanol, fraction 3 (50 : 25 : 1), and (10 : 5 : 1) fractions 4 and 5. The fraction 3 provided 37 mg of the compound *XII*, from the fractions 4 and 5 11 mg of *XI* and 44 mg of *X*, respectively were obtained. The fraction 8 was subjected to the chromatography on the silica gel impregnated by sodium hydrogen carbonate column using the systems benzene–chloroform, chloroform, chloroform–methanol. The fraction containing *IX* yielded after TLC separation on the silica gel impregnated by sodium hydrogen carbonate in the system chloroform–methanol (49 : 1) 29 mg of this compound. All compounds were finaly recrystalized from ethanol. The R_F values of the studied metabolites (TLC on silica gel impregnated by $NaHCO_3$ (10 : 1) in the system chloroform–methanol (49 : 1) were: *II* 0·24, *I* 0·28, *IX* 0·32, *IV* 0·39, *III* 0·44, *X* 0·59, *XI* 0·60, *XII* 0·78, *VI* 0·85 and *V* 0·88.

1,8-*Dihydroxy*-3-*methoxycarbonylmethyl*-2-(1-*oxopropyl*)-9,10-*anthraquinone* (XII): yellow needles, m.p. 162—164°C (ethanol). UV/VIS spectrum: λ_{max} 230, 255, 277 sh, 287, 427 nm. IR spectrum: 3 450, 1 735, 1 685, 1 660, 1 620 cm^{-1}. Mass spectrum: m/z (% of relative intensity, elemental composition, assignment) 368 (22, $C_{20}H_{16}O_7$, M^+), 339 (24, $C_{18}H_{11}O_7$, $M-C_2H_5$), 308 (38, isotopic peak), 307 (100, $C_{17}H_7O_6$, $M-C_2H_5-CH_4O$), 251 (22, $C_{15}H_7O_4$, $M-C_2H_5-CH_4O$ —2 CO), 139 (9, $C_{11}H_7$).

1,5,8-*Trihydroxy*-3-*methoxycarbonylmethyl*-2-(3-*oxopentyl*)-9,10-*anthraquinone* (XI): deep red needles, m.p. 177–179°C (ethanol). UV/VIS spectrum: λ_{max} 237, 261, 292, 297 sh, 487 nm. IR spectrum: 3450, 1720, 1700, 1610 cm^{-1}. Mass spectrum: *m/z* 412 (73, $C_{22}H_{20}O_8$, M^+), 394 (20, $C_{22}H_{18}O_7$, $M-H_2O$), 383 (6, $C_{20}H_{15}O_8$, $M-C_2H_5$), 381 (10, $C_{21}H_{17}O_7$, $M-CH_3O$), 380 (9, $C_{21}H_{16}O_7$, g*), 365 (14, $C_{20}H_{13}O_7$, $M-H_2O-C_2H_5$), 357 (21, isotopic peak), 356 (99, $C_{19}H_{16}O_7$, h), 355 (30, $C_{19}H_{15}O_7$, $M-C_3H_5O$), 335 (27, $C_{20}H_{15}O_5$, $M-H_2O-COOCH_3$), 324 (29, $C_{18}H_{12}O_6$), 323 (56, $C_{18}H_{11}O_6$, i), 321 (13, $C_{19}H_{13}O_5$), 309 (24, $C_{17}H_9O_6$), 296 (43, $C_{17}H_{12}O_5$), 295 (100, $C_{17}H_{11}O_5$, j)), 283 (20, $C_{16}H_{11}O_5$), 281 (33, $C_{16}H_9O_5$, k), 57 (71, C_3H_5O), 29 (69, C_2H_5).

1,8-*Dihydroxy*-3-*methoxycarbonylmethyl*-2-(3-*oxopentyl*)-9,10-*anthraquinone* (X): orange needles, m.p. 176–178°C (ethanol). UV/VIS spectrum: λ_{max} 230, 257, 279 sh, 289, 428 nm. IR spectrum: 3450, 1725, 1700, 1670, 1620 cm^{-1}. Mass spectrum: *m/z* 396 (41, $C_{22}H_{20}O_7$, M^+), 378 (22, $C_{22}H_{18}O_6$, $M-H_2O$), 367 (7, $C_{20}H_{15}O_7$, $M-C_2H_5$), 365 (10, $C_{21}H_{17}O_6$, $M-CH_3O$), 364 (9, $C_{21}H_{16}O_6$, g), 349 (25, $C_{20}H_{13}O_6$, $M-H_2O-C_2H_5$), 341 (23, isotopic peak), 340 (100, $C_{19}H_{16}O_6$, h), 339 (20, $C_{19}H_{15}O_6$, $M-C_3H_5O$), 319 (15, $C_{20}H_{15}O_4$, $M-H_2O-COOCH_3$), 308 (28, $C_{18}H_{12}O_5$), 307 (47, $C_{18}H_{11}O_5$, i), 305 (10, $C_{19}H_{13}O_4$), 293 (20, $C_{17}H_9O_5$), 280 (34, $C_{17}H_{12}O_4$), 279 (72, $C_{17}H_{11}O_4$, j), 267 (14, $C_{16}H_{11}O_4$), 265 (28, $C_{16}H_9O_4$, k), 57 (44, C_3H_5O), 29 (39, C_2H_5).

9-*Ethyl*-1,4,6,7-*tetrahydroxy*-10-*methoxycarbonyl*-7,8-*dihydro*-5,12-*naphthacenequinone* (IX): red-orange crystals provide deep red needles at 162°C which melt at 247–248°C. UV/VIS spectrum: λ_{max} 234, 254, 288, 492 nm. IR spectrum: 3500, 1720, 1610 cm^{-1}. Mass spectrum: *m/z* 410 (1, $C_{22}H_{18}O_8$, M^+), 398 (9, contamination), 393 (26, isotopic peak), 392 (100, $C_{22}H_{16}O_7$, $M-H_2O$), 380 (6), 377 (34), 361 (27), 360 (24), 359 (16), 333 (14), 332 (15), 331 (9), 321 (22), 305 (7), 295 (8).



SCHEME 1

* Ions designed according to the ref.[13].

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