NAPHTHACENEQUINONE DERIVATIVES FROM A MUTANT STRAIN OF *Streptomyces coeruleorubidus*

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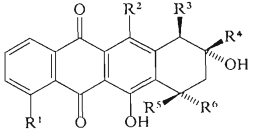
Five metabolites were isolated from a mutant strain of *Streptomyces coeruleorubidus*. Structures *IX—XIII* were proposed on the basis of their ^{1}H and ^{13}C-NMR, IR, UV/VIS and mass spectra.

Streptomyces coeruleorubidus is known as the producer of anthracyclines, glycosidic antibiotics having antitumor activity[1-4]. The aglycone in all compounds so far isolated from this source was either daunomycinone (*I*) or its dihydro derivative *II*. As the other constituents were found ε-rhodomycinone (*III*), compound *II*, its 7-deoxy derivative *IV* and its 7-epimer *V* (refs[4-6]). In the course of the genetic improvement of this strain, we obtained the mutant 24–47 (type E), which did not produce glycosidic compounds and differred from the parent strain by its bright yellow colour[7]. Aklavinone (*VI*) and 7-deoxyaklavinone (*VII*) were identified by thin-layer chromatography as its major metabolites[7]. From the combined extracts of the mycelium and the fermentation broth, we isolated another eight compounds F1–F8. This paper deals with the elucidation of their structures by spectroscopic methods.

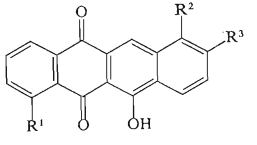
Compounds F2, F5 and F6 were identified by comparison with the authentic samples (chromatographic behaviour, melting point, UV/VIS, IR, mass and ^{1}H-NMR spectra) as aklavinone (*VI*), 7-deoxyaklavinone (*VII*), and bisanhydroaklavinone (*VIII*). With the first two compounds, the identification was confirmed by ^{13}C-NMR spectra.

The compound F4 has an empirical formula $C_{22}H_{20}O_7$ and its mass spectrum differs from that of 7-deoxyaklavinone (*VII*) in the relative intensities of some peaks only (Fig. 1). Its ^{1}H-NMR spectrum, however, besides the features common with that of *VII* (signals of two phenolic hydroxyls, an ABC system of three vicinal aromatic protons H–1, H–2 and H–3, a singlet of an isolated aromatic proton H–11, a three-proton singlet of the methyl ester group, an AA'BB' system of the protons at $C_{(7)}$ and $C_{(8)}$ and characteristic signals of the ethyl group) exhibit also some differences: there is a two-proton doublet instead of an one-proton singlet of H–10 with nearly the same chemical shift and the quartet due to the ethyl group methylene

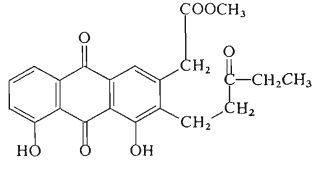
is shifted 0·80 ppm downfield with respect to *VII*. Both the presence of an AA'BB' system of the protons at $C_{(7)}$ and $C_{(8)}$ and the distinct quartet of the ethyl group methylene indicate that $C_{(9)}$ carries no protons. However, its substituent must have greater electronegativity than that of *VII*, in order to explain the observed downfield shifts of the $C_{(8)}$ and $C_{(13)}$ protons in its neighbourhood. The comparison of the ^{13}C-NMR spectra of *VII* and F4 shows that with F4 the singlet (off-resonance multiplicity) at 70·6 ppm assigned to $C_{(9)}$ and the doublet of $C_{(10)}$ at 56·5 ppm are missing. New signals appear – one methylene (triplet) more in the region of 20–40 ppm and a ketone singlet at 210·4 ppm. The infrared spectrum exhibits an OH band (3500 cm^{-1}), two quinone carbonyl bands (1620 and 1670 cm^{-1}), an ester band



	R^1	R^2	R^3	R^4	R^5	R^6
I,	OCH_3	OH	H	$COCH_3$	H	OH
II,	OCH_3	OH	H	$CH(OH)CH_3$	H	OH
III,	OH	OH	$COOCH_3$	CH_2CH_3	H	OH
IV,	OCH_3	OH	H	$CH(OH)CH_3$	H	H
V,	OCH_3	OH	H	$CH(OH)CH_3$	OH	H
VI,	OH	H	$COOCH_3$	CH_2CH_3	H	OH
VII,	OH	H	$COOCH_3$	CH_2CH_3	H	H
XII,	OCH_3	H	H	$CH(OH)CH_3$	H	H



	R^1	R^2	R^3
VIII,	OH	$COOCH_3$	CH_2CH_3
X,	OCH_3	H	CH_2CH_3
XI,	OCH_3	H	$CH(OH)CH_3$
XIII,	OH	H	CH_2CH_3



IX

(1723 cm^{-1}) and another band at 1700 cm^{-1}. From the above mentioned facts it follows that the substituent at $C_{(9)}$ is a keto group and that the ring D is opened The ring C is substituted by a hydroxy group and the side chains $—CH_2CO_2CH_3$ and $—CH_2CH_2COCH_2CH_3$. Starting with the 1,8-dihydroxyanthraquinone system (common both to *VII* and the compound F4), deduced from the similarity of their UV/VIS spectra, we obtain the formula *IX*. The difference of nearly one order of magnitude in the intensities of the *m/z* 340 ions with *VII* and *IX* could be explained by their genesis. With *VII*, this ion is formed after retroaldolization during which the bond between $C_{(9)}$ and $C_{(10)}$ is broken[8] whereas with *IX* it can be formed directly from the molecular ion. The ion *m/z* 319 $M-H_2O—COOCH_3$ is with *VII* of one half order more intense than with *IX* since its formation extends the conjugated system.

The compound F3 has a summary formula $C_{21}H_{16}O_4$, corresponding to a fully aromatic naphthacenequinone skeleton. This conclusion is supported by an intense molecular ion and the UV/VIS spectra. The infrared spectrum contains the bands of hydroxyl group, chelated and unchelated carbonyls. The ^{1}H-NMR spectrum displays a triplet of a primary methyl group at 1·35 ppm, the corresponding quartet

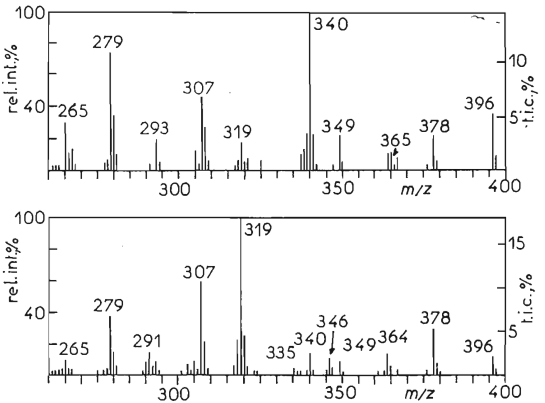


FIG. 1

Mass Spectra of the Compound F4 (*IX*) and 7-Deoxyaklavinone (*VII*)

at 2·89 ppm (J = 7·3 Hz), a three-proton singlet at 4·10 ppm, signals of seven aromatic protons in the region of 7·33–8·81 ppm and a singlet of one phenolic hydroxyl at 14·90 ppm. There are four oxygen atoms in the molecule; two of them are part of the quinone system, one is due to the phenolic hydroxyl. From the balance of these atoms it follows that the signal at 4·10 ppm in the ^{1}H-NMR spectrum cannot be that of $COOCH_3$ group for which one oxygen atom is lacking but must represent an aromatic methoxyl. The chemical shift of the ethyl group methylene indicates that this group is attached to the aromatic nucleus. In the mass spectrum, the presence of the ethyl group is indicated by splitting off the methyl radical from the $M-H_2O$ and $M-H_2O$—CO ions and in smaller extent by the elimination of the ethyl radical from the molecular ion. The presence of the methoxyl is manifested by the elimination of water only, an effect already observed with daunomycinone (*I*) and its dihydro derivative *II* (ref.[9]). Therefore, the naphthacenequinone skeleton is substituted by methoxyl, ethyl, and hydroxy groups. The aromatic protons constitute an ABC system of three vicinal protons at 8·45 dd (J_{ortho} = 7·6 Hz, J_{meta} = = 1·5 Hz), 7·75 t (twice J_{ortho} = 7·6 Hz and 7·37 dd (J_{ortho} = 7·6 Hz, J_{meta} = 1·5 Hz), singlet of one isolated proton at 7·74 ppm and another ABC system corresponding to the 1,3,4-substitution pattern: 8·21 d (J_{meta} = 1·6 Hz, J_{para} = 0), 8·07 dd (J_{ortho} = = 7·3Hz, J_{meta} = 1·6 Hz) and 7·56 d (J_{ortho} = 7·3 Hz, J_{para} = 0). Considering the imposed limitations and the biogenesis, we obtain the formula *X*.

This structure is more probable than the alternatives with hydroxyl at $C_{(11)}$ instead at $C_{(6)}$ or with ethyl attached to the position 8 instead to 9, which are also consistent with the observed spectra.

The most intense ion in the mass spectrum of the compound F7 is the molecular ion with elemental composition $C_{21}H_{16}O_5$. Its degree of unsaturation again indicates the presence of four aromatic rings in the molecule. The UV/VIS spectrum is nearly identical with that of compound *X*. Also the ^{1}H-NMR spectra in the region of aromatic protons are very similar. In the ^{1}H-NMR spectrum, there is a doublet of the secondary methyl group at 1·60 ppm (J = 6·1 Hz), a quartet at 5·12 ppm (J = 6·1 Hz) a three-proton singlet at 4·12 ppm, multiplets of seven aromatic protons in the region 7·23–8·57 ppm and a singlet of one phenolic hydroxyl group at 14·88 ppm. The ^{13}C-NMR spectrum confirms the presence of seven sp^2-type methines 111·7 d, 120·2 d, 120·6 d, 124·9 d, 125·8 d, 126·3 d and 135·4 d, two sp^2-carbons attached to oxygen (158·2 s, 163·1 s) and two quinone carbonyls (one of them hydrogen-bonded) at 186·5 and 191·5 ppm. There are only three signals in the high field region: 21·5 q, 56·5 q and 69·9 d. Selective decoupling proved the direct coupling between the proton at 5·12 ppm and the carbon at 69·9 ppm. This proton is also the methine proton of the secondary methyl group. Its chemical shift together with the above mentioned facts allows us to formulate the partial structure C—$CH(OH)CH_3$. From the balance of the oxygen atoms (two due to quinone, one phenolic hydroxyl, one side chain hydroxyl), follows that the fifth oxygen atom might be a part of an aro-

matic methoxyl group only (δ_H 4·12, δ_C 56·5). Also in this case, the investigated compound is a trisubstituted (OH, OCH_3, and $CH(OH)CH_3$) naphthacenequinone. The molecular ion in the mass spectrum loses two molecules of water (from the side chain[9] and from the methoxyl[10]) or splits off the radical C_2H_3O from the side chain[9]. The later splitting takes place also with the $M-H_2O$ ion. The detailed examination of the signals of aromatic protons reveals a singlet of one isolated proton at 7·75 ppm and two ABC systems. First of them is due to three vicinal protons −8·47 dd (J = 7·5 and J 1·5 Hz), 7·95 t (J = 7·5 Hz) and 7·38 dd (J = 7·5 and J = 1·5 Hz). The second one, similarly to the compound F3, is due to the protons with 1,3,4 relative position: 8·20 d (J = 1·5 Hz), 8·05 dd (J = 7·3 and 1·5 Hz) and 7·69 d (J = 7·3 Hz). Among the anthracyclinone skeletons, the observed data are satisfied by the formula *XI*.

The most polar compound F8 has elemental composition $C_{21}H_{20}O_6$, indicating 12 unsaturations (rings plus double bonds). The ^{1}H-NMR spectrum contains a doublet of the secondary methyl group at 1·28 ppm (J = 6·1 Hz), a quartet of an OCH-type proton at 3·70 ppm (J = 6·1 Hz), a three-proton singlet at 4·08 ppm, the signals of four aromatic protons in the region of 7·25 − 8·08 ppm and a singlet of a phenolic hydroxyl group at 13·39 ppm. Aromatic protons constitute an ABC system of three vicinal protons from which one signal is readily discernible 8·01 dd, (J = 7·3 and 1·5 Hz) and a singlet of one isolated aromatic proton at 7·53 ppm. The above mentioned facts indicate that the fourth ring is alicyclic. The fragmentation under electron impact exhibit triple elimination of water (the hydroxyl from the D-ring, from the side chain and from the methoxyl), simple elimination of the side chain as the radical C_2H_5O from the M^+ ion and the elimination of the side chain as C_2H_4O from the $M-H_2O$ ion with the hydrogen transfer. The last mentioned elimination indicates that the hydroxyl in the D-ring is not attached at the position 7 but at 9. It must be a tertiary hydroxyl since there is no oxymethine proton left in the ^{1}H-NMR spectrum. Incorporating the substituents into the naphthacenequinone skeleton using the analogy with the other compounds, we obtain the formula *XII* for F8.

The less polar compound F1 has a summary formula $C_{20}H_{14}O_4$. The corresponding degree of unsaturation (14), intense molecular ion in the mass spectrum and its UV/VIS spectrum qualify it as a fully aromatic compound. The ^{1}H-NMR spectrum contains the signals of an ethyl group (triplet at 1·37 ppm, quartet at 2·89 ppm, J = 7·3 Hz), multiplets of seven aromatic protons in the region of 7·25 − 8·68 ppm and the singlets of two phenolic hydroxy groups at 12·35 and 13·82 ppm. The chemical shift of the ethyl group quartet indicates that this group is attached to a sp^2-hybridized carbon. This fact is in agreement with the observed splitting of the methyl radical from the molecular ion (α-splitting to an aromatic system). The expulsion of carbon monoxide (mass spectrum) confirms the presence of the anthraquinone system in the molecule. The infrared spectrum brings evidence for the hydroxy group and two quinone carbonyls (chelated and unchelated). From

the total four oxygen atoms, two form the quinone moiety and two are due to the phenolic hydroxyls. In the ^{1}H-NMR spectrum of the aromatic protons, it can be identified one ABC system of three vicinal protons H−1, H−2 and H−3 together with a singlet of an isolated aromatic proton at 7·73 ppm assignable to H−11. The remaining three protons form another ABC system from which only the signal at 8·29 ppm d, (J = 1·5 Hz) is resolved. Since this proton has only one *meta*-coupling, there must be some substitution at the position 8 or 9. The last possibility is usual with anthracyclinones what gives the structure *XIII* for F1 as the most probable alternative.

The comparison of structures of our compounds with the usual metabolites of *S. coeruleorubidus* shows some noteworthy facts. *1*) All compounds isolated from the mutant strain are lacking the hydroxyl at the position 11. Instead of ε-rhodomycinone (*III*) is formed aklavinone (*VI*), instead of compound *IV* is formed the compound *XII*, *etc.* Evidently, the hydroxylation of the aromatic system is blocked. *2*) The proportion of fully aromatic compounds *VIII*, *X*, *XI*, *XIII* increases. Similar overproduction of such compounds was described earlier by Heggyi and Gerber[11]. *3*) The compounds *X* and *XIII* represent the first case of the occurrence of the ethyl side chain in the C_{21}- and C_{20}-anthracyclinones, (*i.e.* in the daunomycinone and carminomycinone series, respectively). *4*) The compound *IX* is a metabolite related to anthracyclinones, having an "incomplete" ring D. It can be eventually their precursor since its aldolization can lead to 7-deoxyaklavinone *VII* or to its isomer. Such reaction was already reported for its pyrromycinone analog[12].

EXPERIMENTAL

The melting points were determined in the Kofler hot stage. Ultraviolet and visible spectra were measured in cyclohexane on a Cary 118C spectrophotometer. Infrared spectra were measured in the KBr pellets on a Unicam SP 200 instrument. Mass spectra were measured on a Varian MAT 311 mass spectrometer (ion source temperature 200°C, direct inlet at 140—200°C, energy of ionizing electrons 11 aJ (70 eV), ionizing current 1 mA). High resolution measurements were performed by peak-matching technique with perfluorokerosene standard (±5 ppm). Metastable ions were recorded in the field-free region between the magnetic and electrostatic sector using the electrostatic field scan. The ^{1}H and ^{13}C-NMR spectra (59·797 and 15·036 MHz) were measured at 25°C in deuteriochloroform with tetramethylsilane as an internal standard on a Jeol FX-60 FT NMR spectrometer. Chemical shifts were calculated from the digitally obtained address differences (accuracy ±0·005 and ±0·06 ppm, respectively). The multiplicity following the ^{13}C-NMR chemical shifts was taken from the off-resonance experiments. The column chromatography was performed on the silica gel Herrman (BRD) 80—200 mesh, saturated by sodium hydrogen carbonate. Further purification was made on the Silufol R^{20} plates (Kavalier Votice, Czechoslovakia). The chromatographic systems were: S1 benzene–chloroform, 1 : 1, S2 *n*-heptane–chloroform–methanol 40 : 60 : 10, S3 *n*-heptane–chloroform–methanol 40 : 60 : 10, S4 *n*-heptane–chloroform–methanol 70 : 25 : 5, S5 benzene–ethyl acetate–methanol 35 : 60 : 5.

Fermentation: The mutant strain of *S. coeruleorubidus* 27—47 (type E)[7] was cultivated five days on a glucose-soya meal medium No. 1 using the reciprocal shaker under the conditions described earlier[5].

Isolation: The mycelium from the whole broth (50 l) was filtered off and extracted by methanol (23 l); the filtrate was extracted by chloroform (3 × 5 l). The solvents were evaporated, dry extracts were combined and washed by light petroleum. Resulting red precipitate (2·8 g) was subjected to column chromatography on silica gel saturated by $NaHCO_3$ in the system S1. Five fractions were obtained, the sixth was eluted by methanol. Preparative chromatography of the first fraction on Silufol R^{20} in S4 yielded 8 mg of the compound F1 (*XIII*). The same work-up of the second fraction provided 21 mg of the compound F2 (*VIII*) and 8 mg of F3 (*X*). The purification of the third fraction by chromatography in S3 afforded 6 mg of *IX*. The separation of the fourth fraction in the systems S3 and S4 yielded 358 mg of 5 (*VII*) and 95 mg of F7 (*XI*). With the sixth fraction, after chromatography in S5, 36 mg of F8 (*XII*) was obtained.

Properties of the Compounds

XII: m.p. 245—247°C; UV/VIS (λ): 260, 267, 283, 401 and 418 nm. Mass spectrum: *m/z* (% of relative intensity, elemental composition, *m/z* of daughter ions): 368 (15, $C_{21}H_{20}O_6$, 350, 323), 350 (29, $C_{21}H_{18}O_5$, 332, 306), 334 (44, $C_{21}H_{18}O_4$, 305), 332 (25, $C_{21}H_{16}O_4$, 314), 323 (100, $C_{19}H_{15}O_4$, 305), 314 (16, $C_{21}H_{14}O_3$), 307 (44, $C_{19}H_{15}O_4$), 306 (58, $C_{19}H_{14}O_4$), 305 (80, $C_{19}H_{13}O_4$, 290), 290 (44, $C_{18}H_{10}O_4$), 287 (31, $C_{19}H_{11}O_3$).

XIII: m.p. 212°C; UV/VIS (λ): 222, 260, 290, 450, and 478 nm; IR: 1608, 1660, and 3480 cm^{-1}. Mass spectrum: 318 (100, $C_{20}H_{14}O_4$, 303), 303 (59, $C_{19}H_{11}O_4$, 275), 275 (18, $C_{18}H_{11}O_3$).

VIII: m.p. 230—232°C; UV/VIS (λ): 244, 257, 263, 290, 447 and 475 nm; Mass spectrum: 376 (100, $C_{22}H_{16}O_6$), 361 (71), 345 (35), 344 (41), 317 (16), 316 (23). ^{1}H-NMR: 1·35 t (*J* = 7·3 Hz, 3 H), 2·85 q (*J* = 7·3 Hz, 2 H), 4·12 s (3 H), 7·30—8·83 mt (6 H), 12·25 s (1 H), 13·76 s (1 H).

X: m.p. 195—196°C; UV/VIS (λ): 256, 288, 305, 432, 452 nm; IR: 1618, 1660, and 3500 cm^{-1}. Mass spectrum: 332 (77, $C_{21}H_{16}O_4$, 314, 303), 314 (100, $C_{21}H_{14}O_3$, 299, 286), 303 (8, $C_{20}H_{15}O_3$ and $C_{19}H_{11}O_4$), 299 (16, $C_{20}H_{11}O_3$), 286 (38, $C_{20}H_{14}O_2$, 271), 271 (22, $C_{19}H_{11}O_2$).

IX: m.p. 182°C UV/VIS (λ): 230, 259, 291, 435, 452 nm. Mass spectrum: 396 (35, $C_{22}H_{20}O_7$), 378 (21), 367 (6), 365 (11), 364 (9), 349 (23), 340 (100), 319 (18), 308 (26), 307 (47), 305 (12), 293 (20), 280 (35), 279 (76), 265 (29). ^{1}H-NMR: 1·06 t (*J* = 7·3 Hz, 3 H), 2·45 q (*J* = 7·3 Hz, 2 H), 2·69—3·14 AA'BB' (4 H). 3·72 s (3 H), 3·90 s (2 H), 7·20—7·90 mt (4 H), 12·05 s (1 H), 12·52 s (1 H). ^{13}C-NMR: 7·80 q, 21·2 t, 35·9 t, 39·4 t, 40·4 t, 52·4 q, 114·4 s, 115·9 s, 119·9 s, 122·3 d, 124·6, 131·1 s, 133·7 s, 137·0 s, 137·2 d, 142·6 s, 161·3 s, 162·4 s, 170·6 s, 180·4 s, 193·0 s, 210·4 s.

VII: m.p. 211—212°C; UV/VIS (λ): 258, 290, 432, 452 nm; Mass spectrum: 396 (11, $C_{22}H_{20}O_7$), 378 (29), 367 (4), 365 (5), 364 (14), 349 (9), 346 (11), 340 (14), 319 (100), 308 (21), 307 (60), 305 (9), 291 (14), 280 (14), 279 (36), 265 (9). ^{1}H-NMR: 1·08 t (*J* = 7·3 Hz, 3 H), 1·65 q (*J* = 7·3 Hz, 2 H), 2·20—3·10 AA'BB' (4 H), 3·73 s (3 H), 3·95 s (1 H), 7·20—7·88 mt (4 H), 12·09 s (1 H), 12·48 s (1 H). ^{13}C-NMR: 6·6 q, 19·9 t, 28·2 t, 32·2 t, 52·5 q, 55·9 d, 71·5 s, 114·2 s, 116·7 s, 119·8 d, 121·1 d, 124·5 d, 126·9 s, 130·7 s, 130·7 s, 137·0 d, 141·9 s, 158·2 s, 160·9 s, 171·4 s, 181·5 s, 192·7 s.

VI: m.p. 167—168°C; UV/VIS (λ): 229, 257, 290, 432 nm. Mass spectrum 412 (2, $C_{22}H_{20}O_8$), 394 (3), 376 (100), 365 (4), 361 (65), 345 (33), 344 (37), 335 (8), 317 (18), 316 (22). ^{1}H-NMR: 1·10 t (*J* = 7·3 Hz, 3 H), 1·67 q (*J* = 7·3 Hz, 2 H), 2·57 dd (*JJ* = 14·7 and 4·9 Hz, 1 H), 2·21 d (2 H), 3·47 exchangeable s (1 H), 3·70 s (3 H), 3·94 exchangeable s (1 H), 4·08 s (1H), 5·37 mt (1 H), 7·13—7·88 mt (4 H), 11·92 s (1 H), 12·68 s (1 H). ^{13}C-NMR: 6·8 q, 32·5 t, 34·8 t, 52·5 q, 56·7 d, 62·5 d, 71·7 s, 114·7 s, 115·8 s, 120·3 d, 121·4 d, 124·9 d, 132·7 s, 132·9 s, 133·6 s, 137·5 d, 142·7 s, 161·3 s, 162·7 s, 171·3 s, 181·3 s, 192·8 s.

XI: m.p. 215—216°C; UV/VIS (λ): 256, 305, 432, 450 nm; IR: 1620, 1660, and 3450 cm^{-1}. Mass spectrum: 348 (100, $C_{21}H_{16}O_5$, 330, 305), 330 (73, $C_{21}H_{14}O_4$, 315, 312, 287), 315 (44,

$C_{20}H_{11}O_4$), 312 (29, $C_{21}H_{12}O_3$), 305 (55, $C_{19}H_{13}O_4$, 290), 290 (47, $C_{18}H_{10}O_4$), 287 (59, $C_{19}H_{11}O_3$). ^{13}C-NMR: 25·1 q, 56·6 q, 69·9 d, 117·9 d, 120·2 d, 120·6 d, 124·9 d, 125·8 d, 126·3 d, 130·1 s, 132·3 s, 135·4 s, 135·9 s, 148·5 s, 158·2 s, 163·1 s, 186·5 s, 191·5 s.

REFERENCES

1. Belg. No 632 391 (1963); Chem. Abstr. *61*, 11 296 (1964).
2. Ger. (GDR) No 1 911 240 (1969); Chem. Abstr. *72*, 20 603 (1970).
3. Brazhnikova M. G., Konstantinova N. V., Pomazkova V. A., Zakharov B. V.: Antibiotiki (Moscow) *11*, 763 (1966).
4. Takahashi Y., Naganawa H., Takeuchi T., Umezawa H., Komiyama T., Oki T., Inui T.: J. Antibiot. *30*, 622 (1977).
5. Blumauerová M., Matějů J., Stajner K., Vaněk Z.: Folia Microbiol. (Prague) *22*, 275 (1977).
6. Matějů J., Vokoun J., Blumauerová M., Vaněk Z.: Folia Microbiol. (Prague) *23*, 246 (1978).
7. Blumauerová M., Stajner K., Pokorný V., Hošťálek Z., Vaněk Z.: Folia Microbiol. (Prague) *23*, 255 (1978).
8. Budzikiewicz H., Djerassi C., Williams D. H.: *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. II, p. 244. Holden Day, San Francisco, London, Amsterdam 1964.
9. Karnetová J., Matějů J., Sedmera P., Vokoun J., Vaněk Z.: J. Antibiot. *29*, 1199 (1976).
10. Bowie J. H., White P. Y.: J. Chem. Soc. B, *1969*, 89.
11. Hegyi J. R., Gerber N. M.: Tetrahedron Lett. *1968*, 1587.
12. Brockmann H.: Fortschr. Chem. Org. Naturst. *21*, 122 (1963).

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