

Streptomyces sp.No.76の生産するジデオキシグリセオロジンCの構造解析

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Identification of Dideoxygriseorhodin C Produced by a *Streptomyces* sp. No.76*¹

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Among two pigments obtained from an actinomycete, *Streptomyces* sp. No.76, one pigment is identified previously as known griseorhodin C while other pigment is unidentified. In this connection, various spectral analyses were conducted to elucidate a structure of other pigment. The other pigment purified was identified as a 7,8-dideoxy derivative of griseorhodin C by the analyses of nuclear magnetic resonance (NMR) spectra and mass (MS) spectra and named dideoxygriseorhodin C.

In a series of our study on searching for new pigments as food color, a microbe producing red pigment, *Streptomyces* sp. No.76 was isolated from a soil sample. We isolated two pigments from culture broths, one of which was previously

identified as griseorhodin C¹⁾ and the other was identified as a new derivative of griseorhodin C and named dideoxygriseorhodin C (2). In the present paper, we report the structural elucidation of pigment 2 (Fig.1).

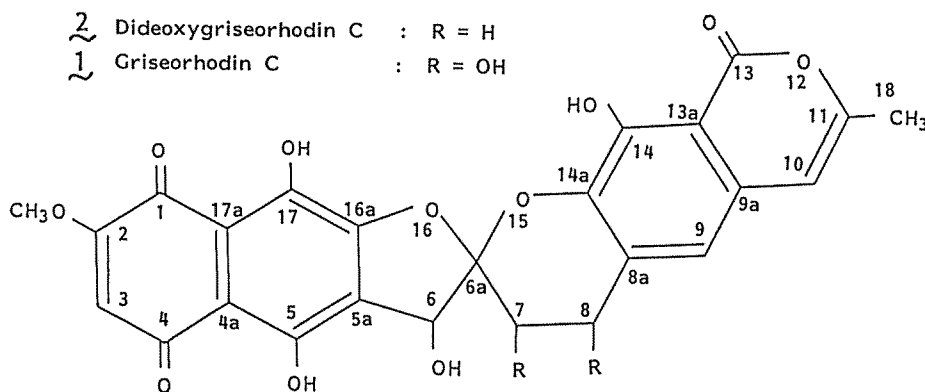


Fig. 1. Structure of griseorhodin C and dideoxygriseorhodin C.

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Material and Methods

Production and Isolation of pigment 2

The strain *Streptomyces* sp. No.76 was cultured at 28°C for 3 days on a rotary shaker in 500 ml Erlenmyer flasks containing 100ml of a medium consisting of 2.5% soluble starch, 0.8% yeast extract, 0.5% polypeptone and 0.5% NaCl (pH7.2). The mycelial cake was obtained by centrifuging the cultured broth (10 ℓ). Crude pigment was extracted from the cake with ethyl acetate in an acidic condition. A flow diagram for the isolation procedures is given in Fig.2 where the final treatment by reversed phase HPLC gave purified pigment 2 (420 mg).

Methods of Analysis

Melting point was determined by Yazawa micro-apparatus. Mass spectra were measured on a Shimadzu GCMS 9020-DF spectrometer, UV and visible spectra on a Shimadzu UV-300 spectrophotometer, Infrared (IR) spectra on a Shimadzu FTIR-4000 spectrophotometer and NMR spectra on a JEOL GX-500 spectrometer with ^1H -

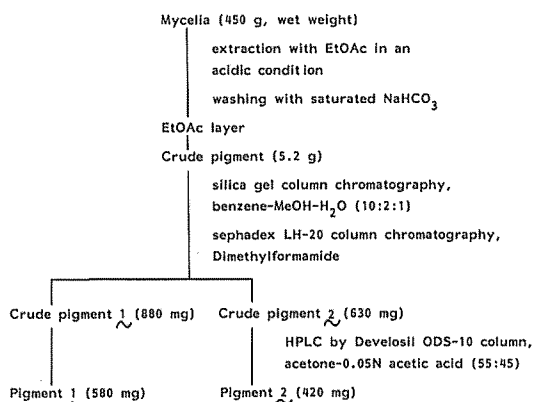


Fig. 2. Isolation procedure for pigment.

NMR at 500 MHz and ^{13}C -NMR at 125 MHz. ^{13}C - ^1H COSY NMR spectrum was obtained on a JEOL GX-270 spectrometer with ^1H -NMR at 270 MHz and ^{13}C -NMR at 67.8 MHz. Chemical shifts are given in ppm using TMS as an internal standard.

Results and Discussion

Pigment 2 has the same physico-chemical

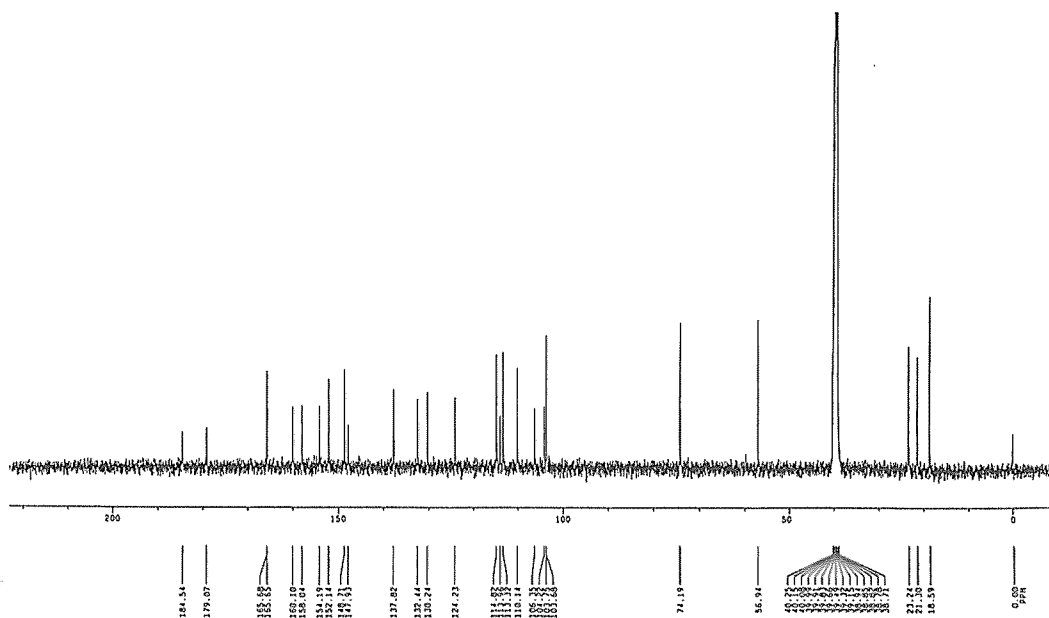


Fig. 3. ^{13}C -NMR spectrum of pigment 2 in $\text{DMSO}-d_6$ at 125 MHz.

properties as pigment 1 (griseorhodin C) previously reported.¹⁾ Pigment 2 melted at 261–264°C with decomposition. The molecular formula was determined by means of mass spectral and elemental analyses. The FD-MS spectrum showed the molecular ion peak at m/z 494 and the high-resolution EI-MS (obs. m/z 494.0870, calcd. 494.0847). Elemental analysis; Found: C, 59.7 H, 3.86 Calcd. for $C_{25}H_{18}O_{11}$; C, 60.7, H, 3.64%. The UV spectrum and the IR spectrum of pigment 2 were very similar to pigment 1 (griseorhodin C) previously reported¹⁾ and showed the presence of a similar chromophore in the molecule.

In the ^{13}C -NMR spectrum (Fig.3), the presence of quinone (α , β , α' , β' -unsaturated) CO is derived from the carbon signals at δ_C 184.54 and δ_C 179.07. No IR absorption band

is observed in the vicinity of $1700\sim 1760\text{ cm}^{-1}$. Therefore, it is difficult to consider that partial structure has chain-type $C=C-COO-$ (1720 cm^{-1}), $-COO-C=O$ (1760 cm^{-1}) and saturated $-COOH$ (1735 cm^{-1}). On the other hand, the IR spectrum shows strong absorption at 1610 cm^{-1} and weak absorption at 1650 cm^{-1} . The 1H -NMR spectrum (Fig.4) at δ_H 11.85 and δ_H 13.24 shows two kinds of the phenolic OH having an intramolecular hydrogen bond. It is, therefore, supported that a quinone structure has an intramolecular hydrogen bond. The Long-range selective proton decoupling (LSPD) experiment established that the proton at δ_H 6.38 is 3J distant from quinone CO (δ_C 179.07). The nuclear Overhauser enhancement (NOE) is observed between δ_H 6.38 and methoxy group at δ_H 3.89. Therefore, the following structure is estimated.

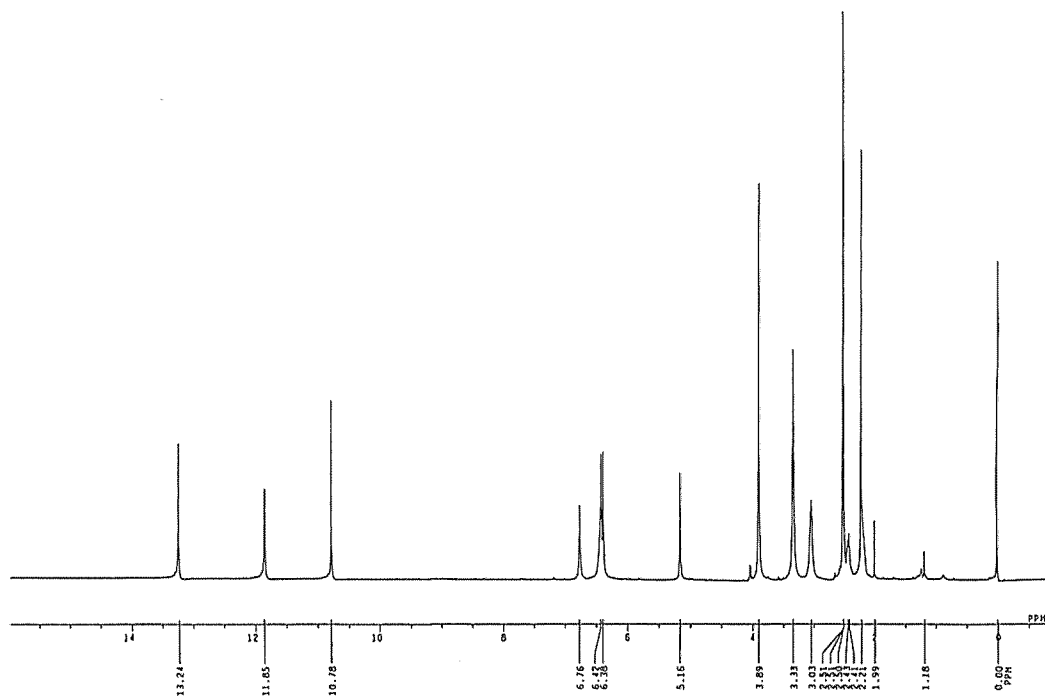
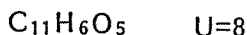
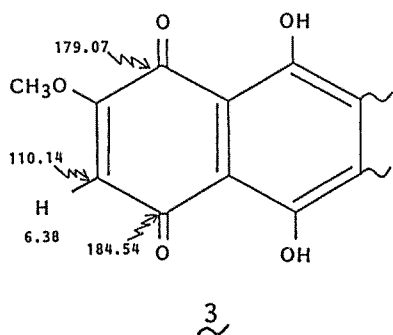
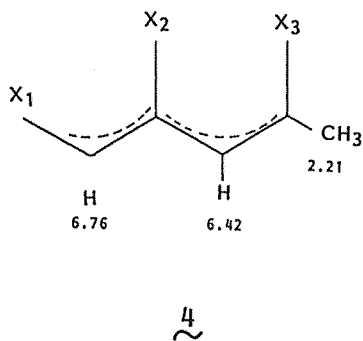


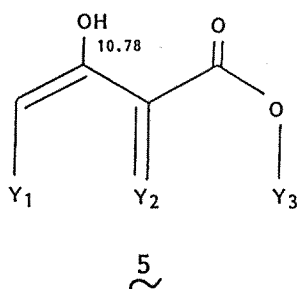
Fig. 4. 1H -NMR spectrum of pigment 2 in $DMSO-d_6$ at 500 MHz.



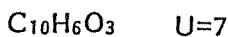
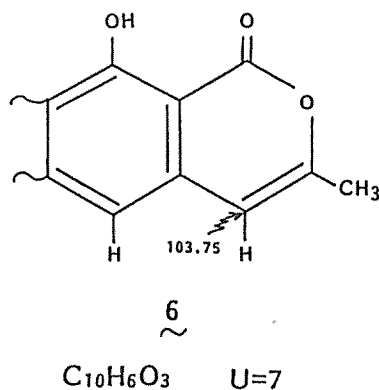
The NOE was observed between methyl proton at δ_{H} 2.21 and methine proton at δ_{H} 6.42, and between δ_{H} 6.42 and δ_{H} 6.76. However, no coupling among these protons is observed. Furthermore, one methyl proton δ_{H} 2.21 and two methine protons δ_{H} 6.42 and δ_{H} 6.76 are combined with sp^2 carbon. Therefore, the following structure is estimated.



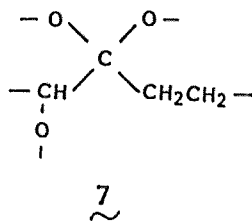
The IR spectrum showed absorption bands at 1690 cm^{-1} and 1240 cm^{-1} . Therefore, it is considered that the partial structure may be not a chain ester but unsaturated lactone structure having an intramolecular hydrogen bond. The hydrogen-bonded phenolic OH can be observed in the $^1\text{H-NMR}$ spectrum at δ_{H} 10.78 and thus the following structure 5 is estimated.



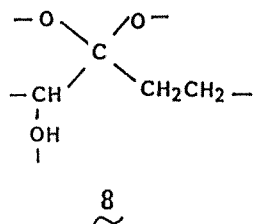
Combination of structure 4 and 5 leads to structure 6. The structure 6 may be supported from the fact that the $^{13}\text{C-NMR}$ chemical shift neighbouring the methyl group is δ_{C} 103.75.



The partial structure which have not been assigned are summarized as follows; $-\text{CH}_2\text{CH}_2 \times 1$, $\text{CH}(\text{O})- \times 1$, $\text{C} \times 2$ and $\text{H} \times 1$. The $^1\text{H-NMR}$ chemical shift of the methylene group is δ_{H} 1.99 and δ_{H} 2.51, and the remaining one carbon different from sp^2 -hybridized one has more downfield shift than δ_{C} 100. Therefore, it is considered to be a spiroketal carbon conjugated two oxygens.



The sum of bond members of structure **3** and **6** is four and that of structure **7** is five. Therefore, it is considered that the partial structure is not -CH(O)- but -CH(OH)-. It is impracticable to consider a proper structural formula for quaternary carbon -OH bond. The structure **8** is therefore estimated.



As described above, the structure formula consisting of a combination of **3**, **6** and **8** can be estimated. It is considered that the typical compound associated with these structures belongs to griseorhodin group.

A 2D J correlated map (COSY), and the accompanying ^1H - and ^{13}C -NMR chemical shifts on the x and y axes are shown in Fig.5. The non-aromatic protons could be unambiguously assigned to mutual connection of those carbons. Especially the chemical shift of C-7 correlated with H-7 and the chemical shift of C-8 correlated with H-8. Therefore, in consideration of the above results it is supported that the pigment **2** is 7,8-dideoxy

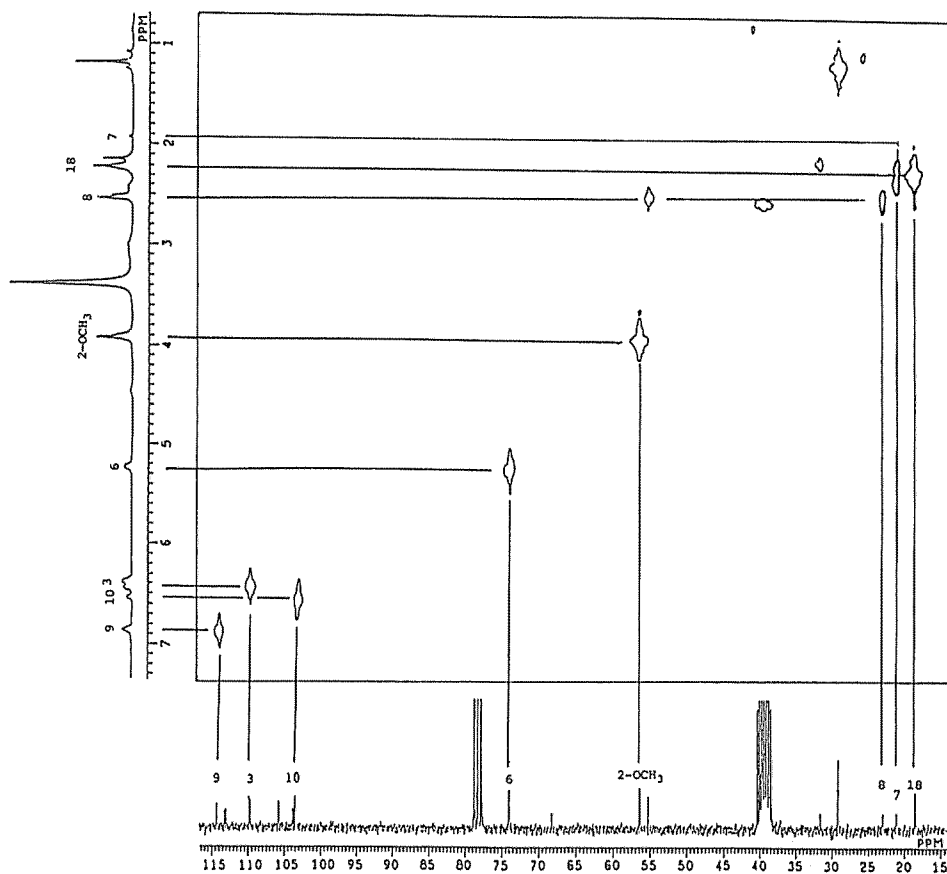


Fig. 5. ^{13}C - ^1H COSY NMR spectrum of pigment **2** in $\text{DMSO}-d_6$ at 270 MHz and 67.8 MHz.

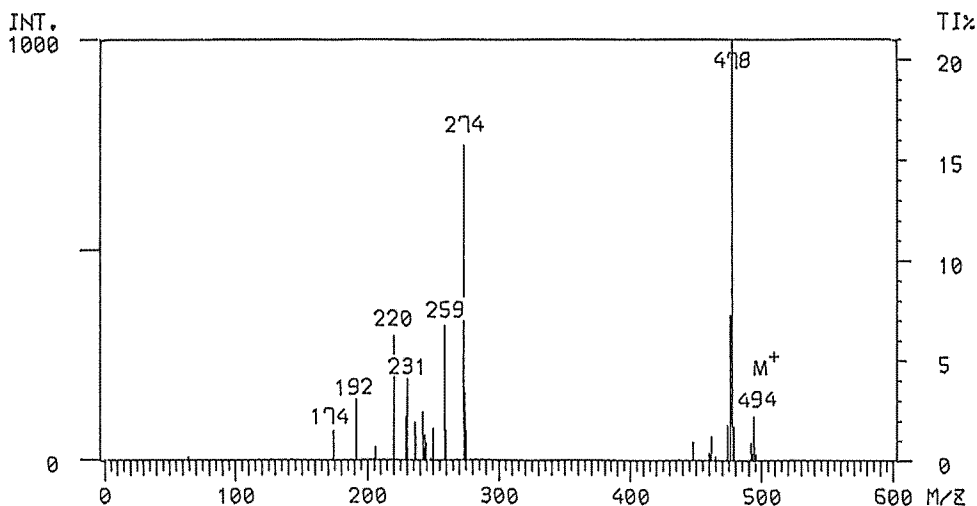


Fig. 6. EI-MS spectrum of pigment 2.

compound.

The molecular ion in the mass spectrum of pigment 2 appeared at m/z 494 and several of the diagnostic ions are rationalized in Figs. 6 and 7. These fragmentation patterns observed for pigment 2, lead the conclusion that the reduction at the chemical shift of C-7 and C-8 should give rise to an important ion at m/z 220 and 274 (the base peak). The diagnostically important fragment ions at m/z 220 (the base peak), 192 (-CO) and 174 (-H₂O) observed for griseorhodin group^{2,3)} and the other fragment ion at m/z 274 compare well to a similar type compound, fusarubin.⁴⁾ Although the stereochemistry of pigment 2 is still undetermined, the structure of pigment 2 has been elucidated to be dideoxygriseorhodin C by the spectral analyses.

Acknowledgments

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References

- 1) K. Suetsuna and Y. Osajima: *J. Shimonoseki Univ. Fish.*, **37**, 51~61 (1988).
- 2) R.M. Stroshane, J.A. Chan, E.A. Chan, E.A. Rubalicaba, A.L. Garretson, and A.A. Aszalos: *J. Antibiotics*, **32**, 197-204 (1979).
- 3) D. Tresselt, K. Eckardt, and W. Ihn: *Tetrahedron*, **34**, 2693-2699 (1978).
- 4) B.E. Cross, P.L. Myers, and G.R.B. Webster: *J.Chem. Soc., (C)*, 930 (1970).

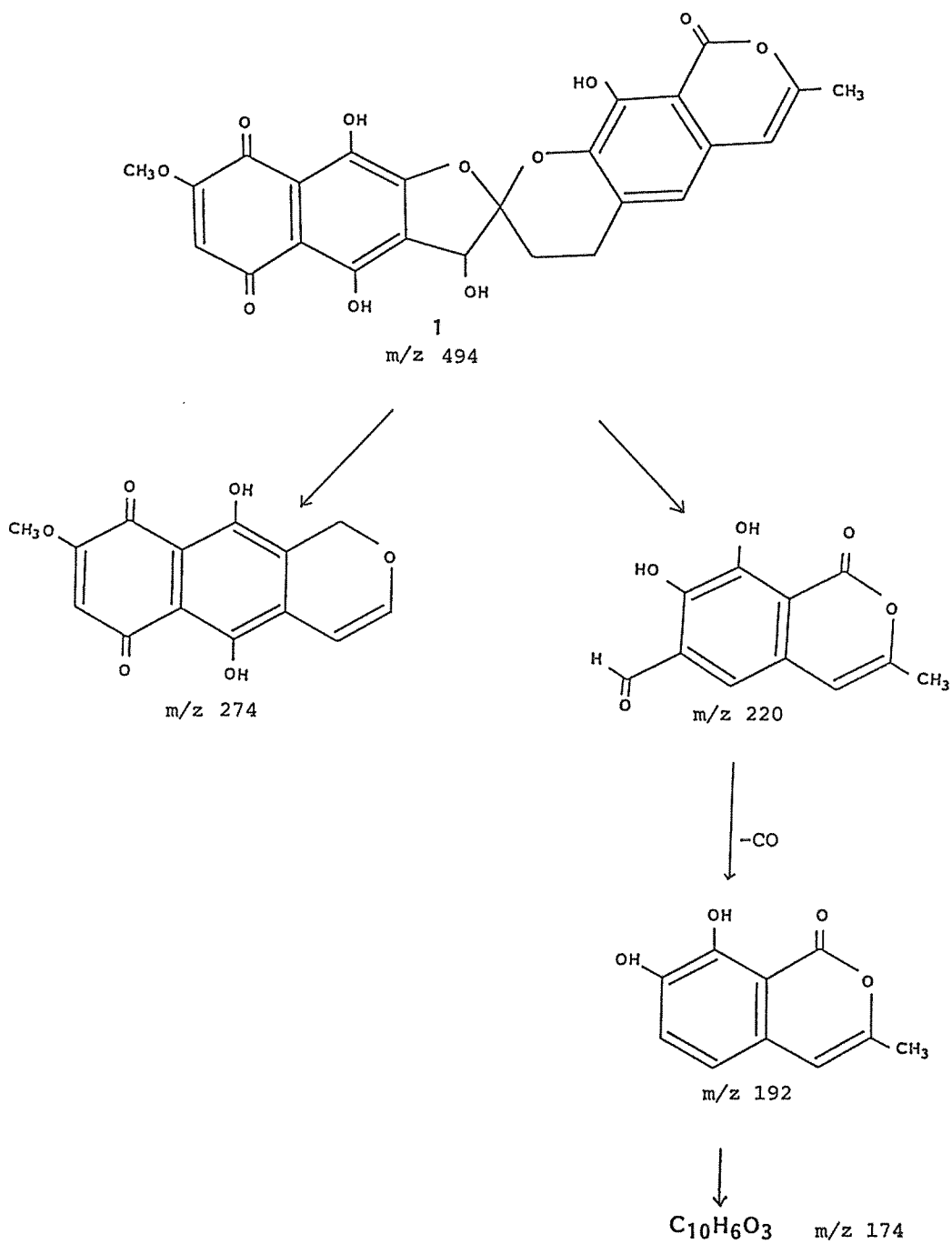


Fig. 7. Mass spectral fragmentations pattern of pigment 2.

Table 1. ^1H - and ^{13}C -NMR assignments of dideoxygriseorhodin C

Position	^1H -	^{13}C -
1		179.07
2		160.10
3	6.38	110.14
4		184.54
4 a		106.35
5		158.04
50H	13.24	
5 a		130.24
6	5.16	74.19
6 a		104.22
7	1.99	21.30
8	2.51	23.24
8 a		124.23
9	6.76	114.82
9 a		137.82
10	6.42	103.75
11		113.32
13		165.68
13 a		132.44
14		152.14
140H	10.78	
14 a		147.93
16 a		148.71
17		154.19
170H	11.85	
17 a		113.96
18	2.21	18.59
2-OCH ₃	3.89	56.94

Chemical shifts are in ppm (δ) downfield from internal TMS in DMSO- d_6 .

Streptomyces sp.No.76 の生産するジデオキシグリセオロジン C の構造解析

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放線菌 *Streptomyces* sp.No.76 の生産する 2 種類の赤色々素のうち、一方については、グリセオロジン C と既に同定したが、ここでは他方の赤色々素の構造解析を行った。発色系に関して UV から色素の母核はグリセオロジンと認められた。一方、高分解能 EI-MS 及び元素分析値より分子式は $\text{C}_{25}\text{H}_{18}\text{O}_{11}$ であり、また、 ^1H -NMR, ^{13}C -NMR, 2D-COSY NMR 及び MS スペクトルよりグリセオロジン C の 7, 8 -ジデオキシ誘導体であると同定した。