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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 previuosly identified as griseorhodin C[1] 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).

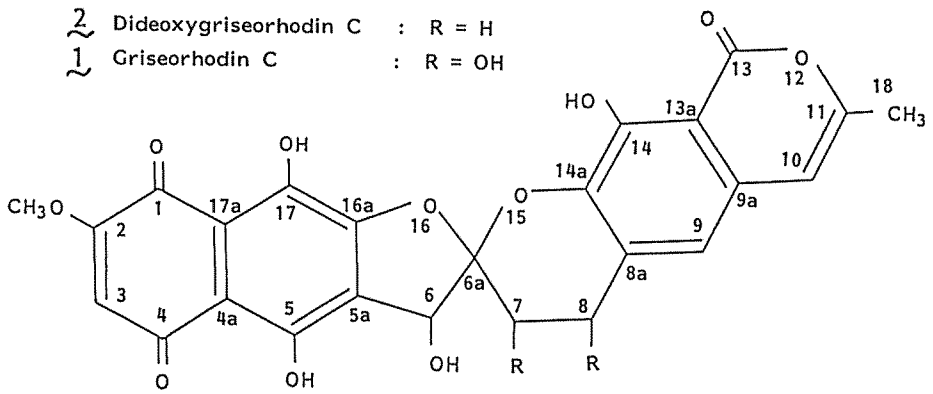

Fig. 1. Structure of griseorhodin C and dideoxygriseorhodin C.

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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 m*l* Erlenmyer flasks containing 100m*l* 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).

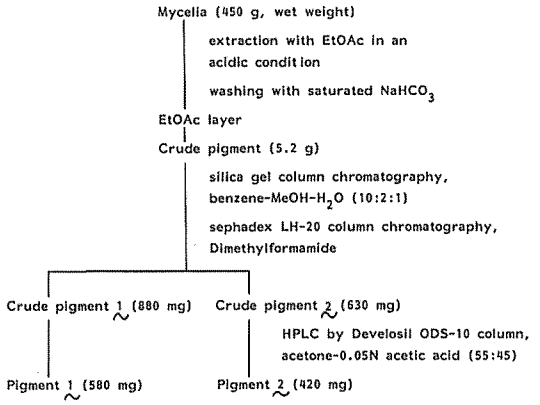


Fig. 2. Isolation procedure for pigment.

Methods of Analysis

Melting point was determined by Yazawa micro-apparatus. Mass spectra were measured on a Shimazu GCMS 9020-DF spectrometer,UV and visible spectra on a Shimazu UV-300 spectrophotometer, Infraded (IR) spectra on a Shimazu FTIR-4000 spectrophotometer and NMR spectra on a JEOL GX-500 spectrometer with ^{1}H-NMR at 500 MHz and ^{13}C-NMR at 125 MHz. ^{13}C-^{1}H COSY NMR spectrum was obtained on a JEOL GX-270 spectrometer with ^{1}H-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

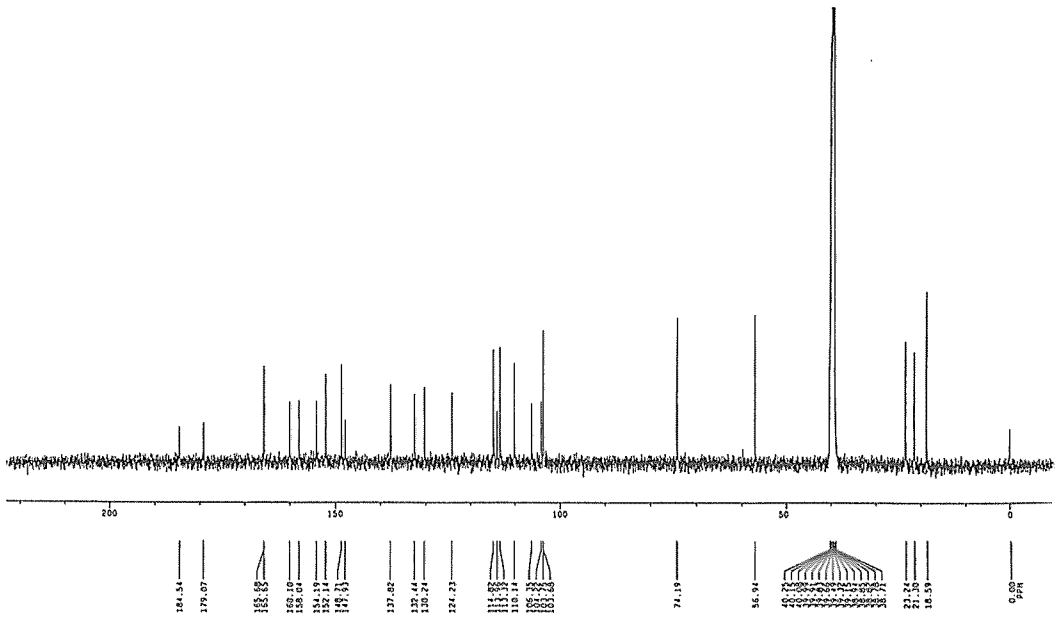


Fig. 3. ^{13}C-NMR spectrum of pigment **2** in DMSO-d_6 at 125 MHz.

properties as pigment **1** (griseorhodin C) previously reported.[1] Pignent **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 reported[1] 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 sbsorption band is observed in the vicinity of 1700～1760 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 ^{1}H-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.

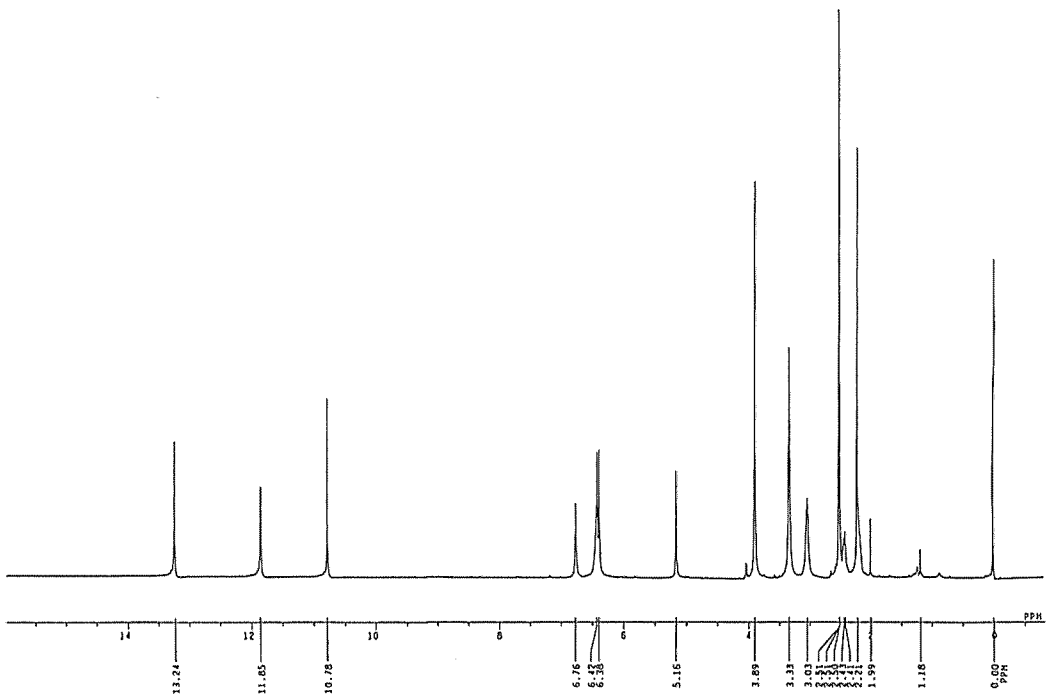
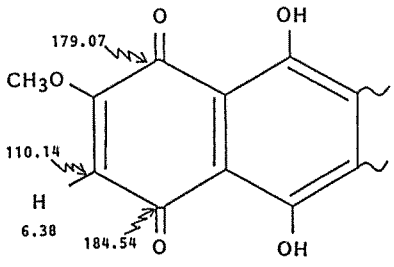


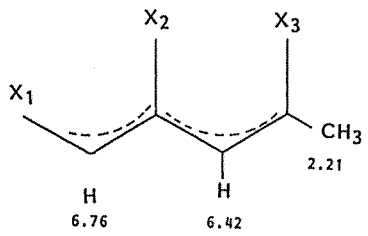
Fig. 4. ^{1}H-NMR spectrum of pigment **2** in DMSO-d_6 at 500 MHz.



3

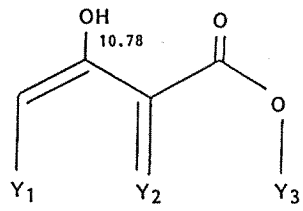
$C_{11}H_6O_5$ U=8

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.



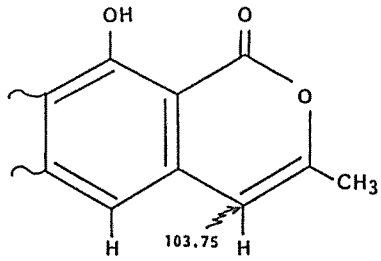
4

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 a intramolecular hydrogen bond. The hydrogen-bonded phenolic OH can be observed in the ^{1}H-NMR spectrum at δ_H 10.78 and thus the following structure **5** is estimated.



5

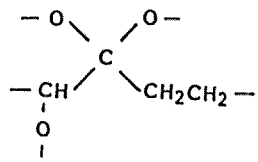
Combination of structure **4** and **5** leads to structure **6**. The structure **6** may be supported from the fact that the ^{13}C-NMR chemianl shift neighbouring the methyl group is δ_C 103.75.



6

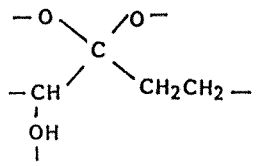
$C_{10}H_6O_3$ U=7

The partial structure which have not been assigned are summarized as follows; $-CH_2CH_2$ × 1, CH(O)- × 1, C × 2 and H × 1. The ^{1}H-NMR chemical shift of the methlene group is δ_H 1.99 and δ_H 2.51, and the remaining one carbon different from sp^2-hybrized one has more downfield shift than δ_C 100. Therefore, it is considered to be a spiroketal carbon conjuated two oxygens.



7

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.



8

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 ^{1}H- and ^{13}C-NMR chemical shifts on the x and y axes are shown in Fig.5. The non-aromatic protons could be unambigously 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

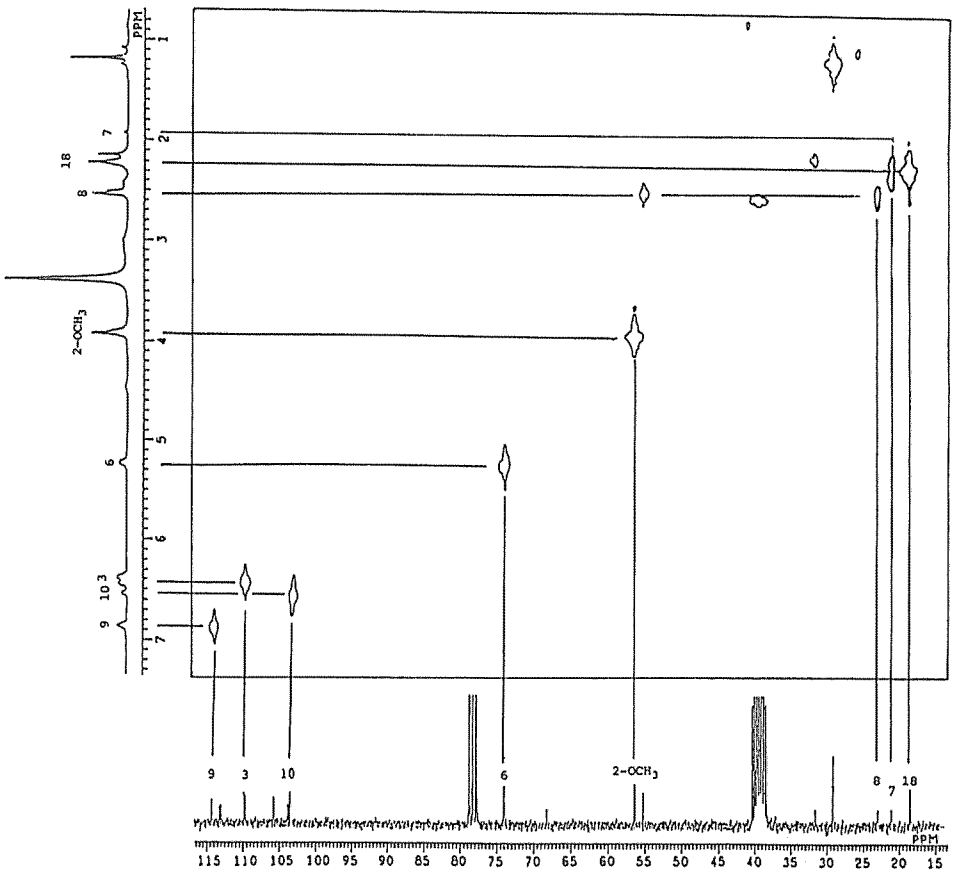


Fig. 5. ^{13}C-^{1}H COSY NMR spectrum of pigment **2** in DMSO-d_6 at 270 MHz and 67.8 MHz.

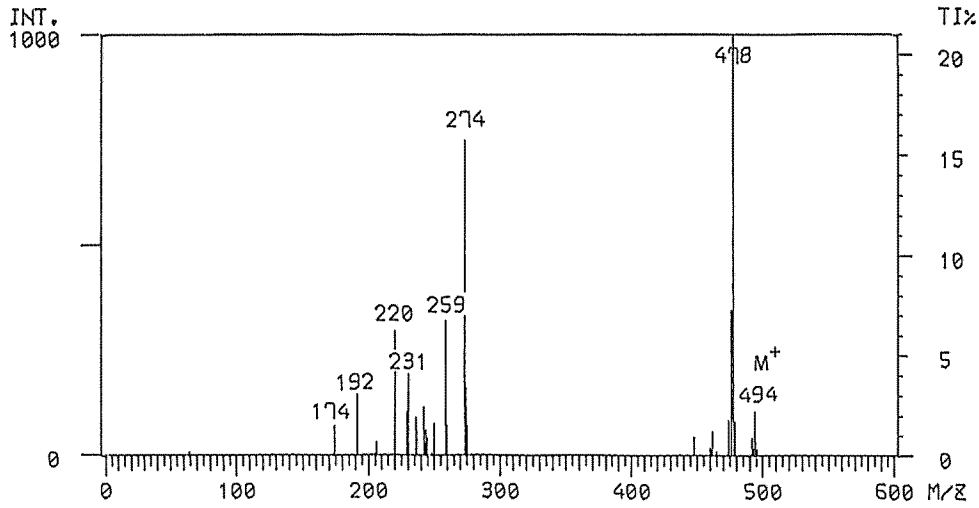


Fig. 6. EI-MS spectrum of pigment **2**.

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 diagnotically important fragment ions at m/z 220 (the base peak),192 (-CO) and 174 ($-H_2O$) 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 streochemistry of pigment **2** is still undetermined, the structure of pigment **2** has been elucidated to be dideoxygriseorhodin C by the spectral analyses.

Acknowledgments

We are indebted to Dr. T. Kondo of Chemical Instrument Center, Nagoya University, for measuring the NOE and LSPD spectra on a JEOL GX-500. We wish to thank Dr. K. Eckardt of Akademie der Wissenschaften der DDR for providing us with an authentic griseorhodin C.

References

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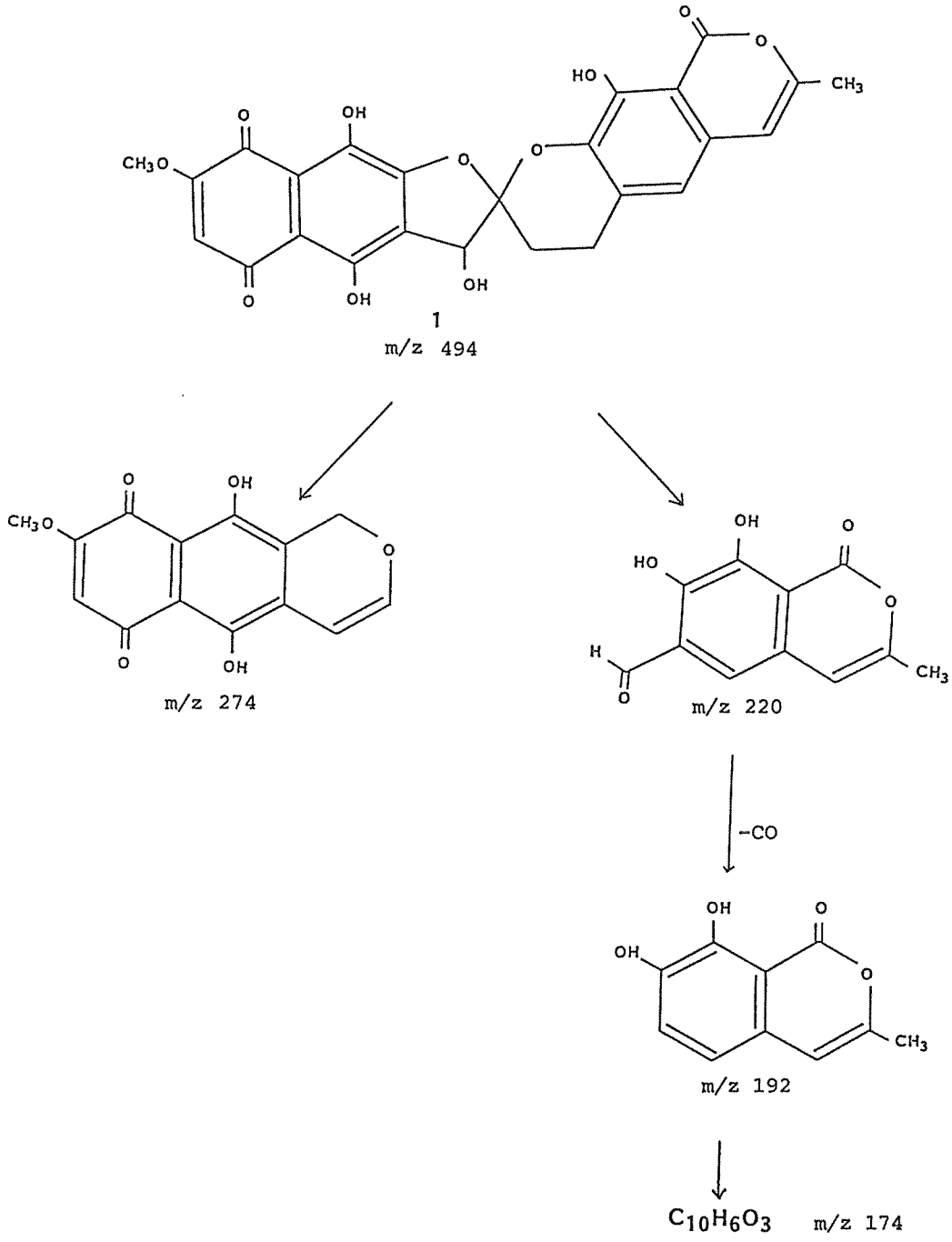

Fig. 7. Mass spectral fragmentations pattern of pigment 2.

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

Position	^{1}H–	^{13}C–
1		179.07
2		160.10
3	6.38	110.14
4		184.54
4 a		106.35
5		158.04
50 H	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
140 H	10.78	
14 a		147.93
16 a		148.71
17		154.19
170 H	11.85	
17 a		113.96
18	2.21	18.59
2-OCH3	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 $C_{25}H_{18}O_{11}$ であり，また，^{1}H-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。