

ACTINOPYRONES A, B AND C, NEW PHYSIOLOGICALLY  
ACTIVE SUBSTANCES

II. PHYSICO-CHEMICAL PROPERTIES AND  
CHEMICAL STRUCTURES

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The structure of physiologically active substances, actinopyrones A, B and C, produced by *Streptomyces pactum* S12538 were determined on the basis of their spectral and chemical character. These substances were structurally related to piericidin A<sub>1</sub>.

Actinopyrones A (**1**), B (**2**) and C (**3**), new physiologically active substances, were isolated from the culture broth of *Streptomyces pactum* S12538 as described in the preceding paper<sup>1)</sup>. This paper is concerned with the physico-chemical properties and chemical structures of **1**, **2** and **3**.

The physico-chemical properties of actinopyrones (**1**, **2** and **3**) are summarized in Table 1. They are neutral colorless oils. They were soluble in common organic solvent and insoluble in water. They

were sensitive to oxidation under air. They were visualized by spraying with 2,4-dinitrophenylhydrazin reagent (yellow) and with anisaldehyde sulfuric acid (purple) and were negative to ferric chloride reaction.

The UV, IR and <sup>1</sup>H NMR spectra of **1** are shown in Figs. 1, 2 and 3, respectively.

High resolution chemical ionization mass spectrometry (CI-MS, CH<sub>4</sub>) on **1**, **2** and **3** established the molecular formulas of these substances as C<sub>25</sub>H<sub>30</sub>O<sub>4</sub>, C<sub>24</sub>H<sub>34</sub>O<sub>4</sub> and C<sub>26</sub>H<sub>38</sub>O<sub>4</sub>, respectively.

Fig. 1. UV spectrum of **1** (MeOH).

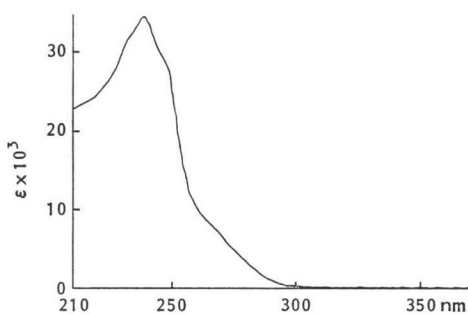
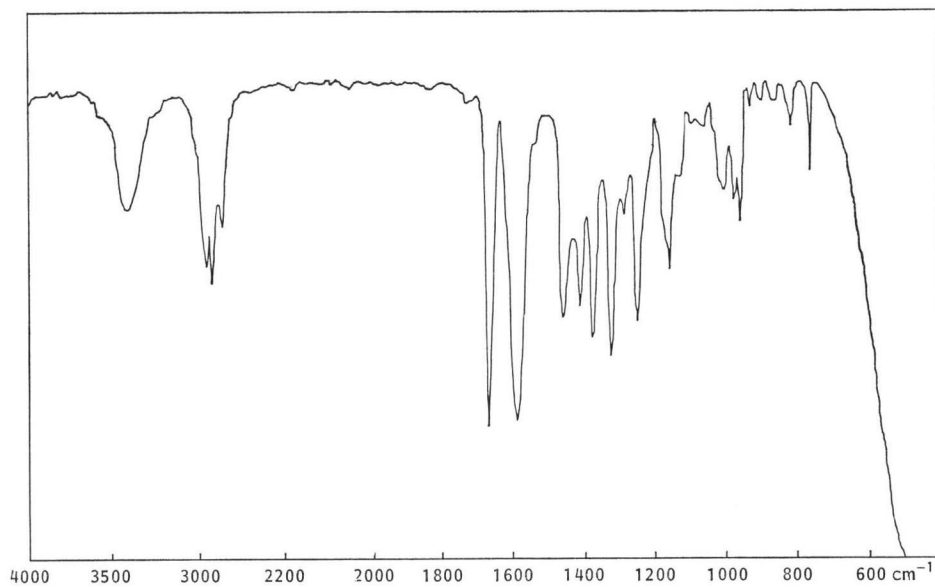
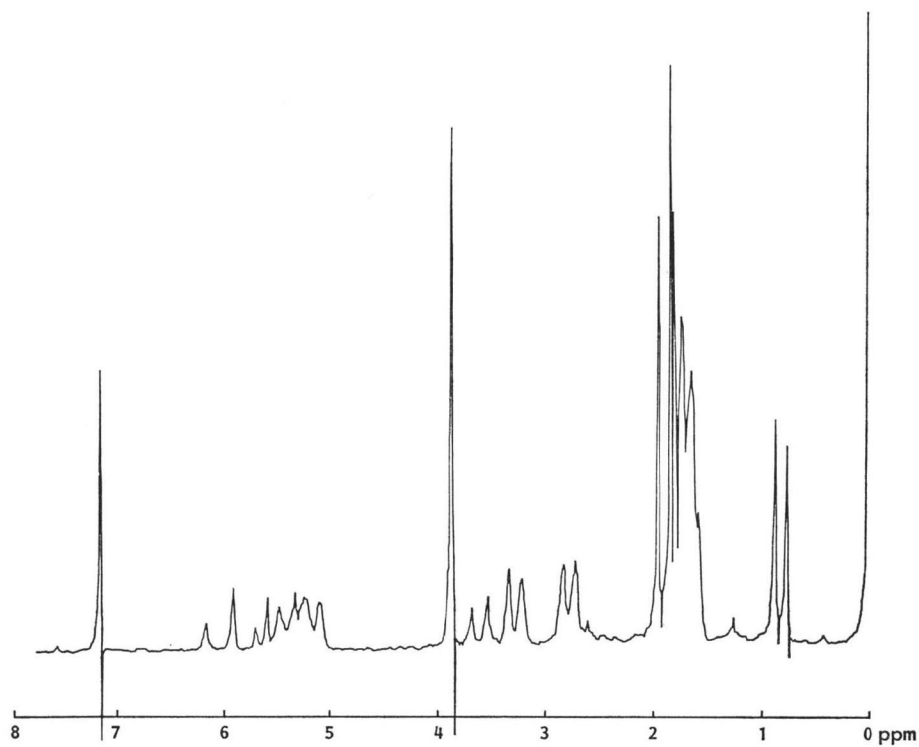


Table 1. Physico-chemical properties of **1**, **2** and **3**.

|  | <b>1</b>   | <b>2</b>   | <b>3</b>   |
|--|--|--|--|
| Appearance   | Colorless oil  | Colorless oil  | Colorless oil  |
| Molecular formula                                      | C <sub>25</sub> H <sub>30</sub> O <sub>4</sub>                     | C <sub>24</sub> H <sub>34</sub> O <sub>4</sub>                     | C <sub>26</sub> H <sub>38</sub> O <sub>4</sub>                     |
| High resolution CI-MS <i>m/z</i><br>(CH <sub>4</sub> ) | 401.2668<br>(M+H, C <sub>25</sub> H <sub>37</sub> O <sub>4</sub> ) | 387.2511<br>(M+H, C <sub>24</sub> H <sub>35</sub> O <sub>4</sub> ) | 415.2583<br>(M+H, C <sub>26</sub> H <sub>39</sub> O <sub>4</sub> ) |
| UV λ <sub>max</sub> <sup>MeOH</sup> nm (ε)             | 239 (33,800)   | 239 (39,000)   | 239 (34,800)   |
| IR ν <sub>max</sub> <sup>film</sup> cm <sup>-1</sup>   | 3400, 1660, 1590   | 3420, 1660, 1590   | 3420, 1660, 1585   |
| Rf value <sup>a</sup>                                  | 0.48   | 0.19   | 0.57   |

<sup>a</sup> Silica gel TLC plate: Merk Art. 5715.  
Solvent: CHCl<sub>3</sub> - EtOAc, 4: 1.

Fig. 2. IR spectrum of **1** (film).Fig. 3. <sup>1</sup>H NMR spectrum of **1** (60 MHz, CDCl<sub>3</sub>).

#### Chemical Structure of **1**

The UV spectrum of **1** resembled to that of piericidin A<sub>1</sub><sup>2)</sup> (**4**). The <sup>1</sup>H NMR of **1** was very similar to that of **4**<sup>3)</sup>. It was noteworthy that the <sup>1</sup>H NMR spectrum of **1** showed only one methoxyl

Fig. 4. Structures of 1, 2, 3 and 4.

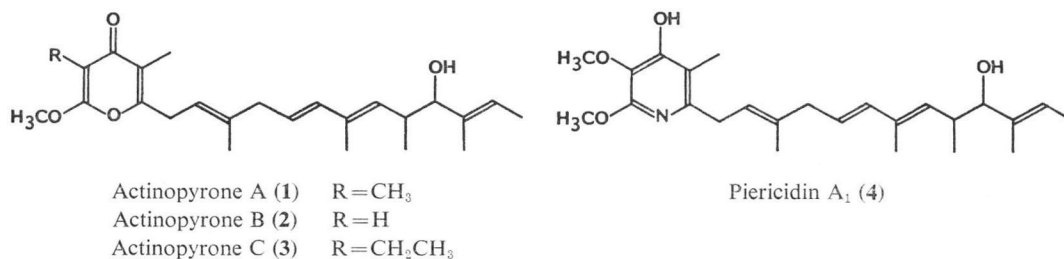
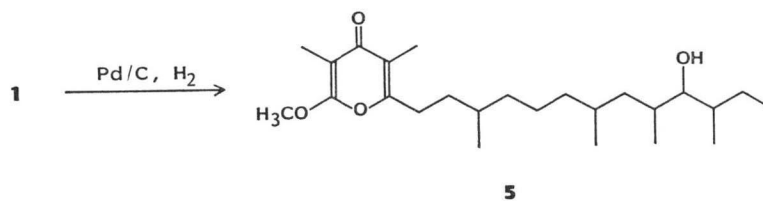


Fig. 5.

Table 2. Comparison of the <sup>13</sup>C NMR data\* for the side chain of 1, 2, 3 and 4.<sup>4)</sup>

| No. | 4         | 1         | 2         | 3         |
|-----|-----------|-----------|-----------|-----------|
| 6   | 34.5 (t)  | 29.9 (t)  | 30.1 (t)  | 30.0 (t)  |
| 7   | 122.4 (d) | 117.9 (d) | 117.8 (d) | 118.0 (d) |
| 8   | 134.7 (s) | 138.0 (s) | 138.1 (s) | 138.0 (s) |
| 9   | 43.2 (t)  | 42.8 (t)  | 42.9 (t)  | 42.9 (t)  |
| 10  | 126.7 (d) | 125.0 (d) | 125.2 (d) | 125.3 (d) |
| 11  | 135.9 (d) | 136.5 (d) | 136.4 (d) | 136.5 (d) |
| 12  | 134.7 (s) | 135.1 (s) | 135.2 (s) | 135.3 (s) |
| 13  | 133.2 (d) | 134.0 (d) | 133.9 (d) | 134.0 (d) |
| 14  | 37.0 (d)  | 36.8 (d)  | 36.8 (d)  | 36.8 (d)  |
| 15  | 82.9 (d)  | 82.6 (d)  | 82.6 (d)  | 82.6 (d)  |
| 16  | 135.9 (s) | 135.9 (s) | 135.9 (s) | 135.8 (s) |
| 17  | 123.3 (d) | 122.9 (d) | 122.9 (d) | 123.1 (d) |
| 18  | 13.1 (q)  | 13.0 (q)  | 13.1 (q)  | 13.1 (q)  |
| 19  | 10.7 (q)  | 10.6 (q)  | 10.6 (q)  | 10.6 (q)  |
| 20  | 17.5 (q)  | 17.4 (q)  | 17.4 (q)  | 17.5 (q)  |
| 21  | 16.6 (q)  | 16.5 (q)  | 16.5 (q)  | 16.5 (q)  |
| 22  | 13.1 (q)  | 13.0 (q)  | 13.1 (q)  | 13.1 (q)  |

\* Measured in CDCl<sub>3</sub> at 22.5 MHz with TMS as an internal standard.

s: Singlet, d: doublet, t: triplet, q: quartet.

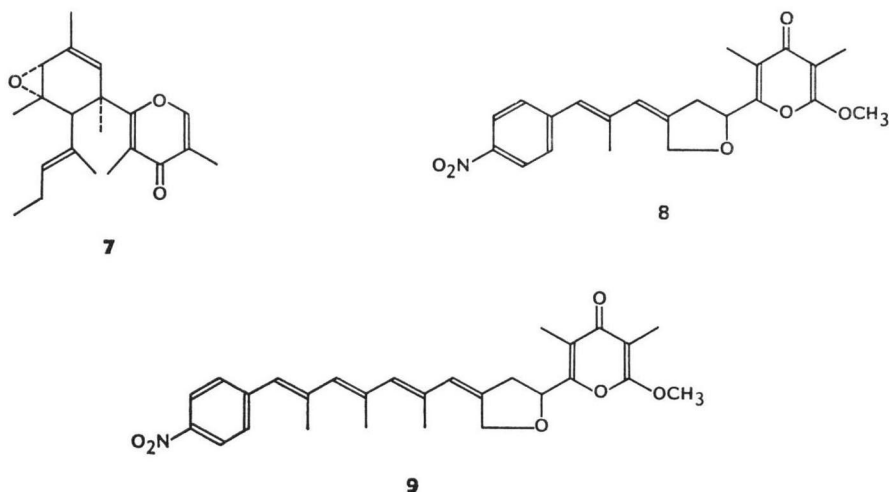
signal while all piericidins<sup>4)</sup> have two or three methoxyl groups. The above data showed that **1** is a new physiologically active substance structurally related with **4**.

When comparing to the <sup>13</sup>C NMR data of **1** with that of **4**, the presence of the same side chain in both substance was evident (Table 2). The comparison of <sup>1</sup>H NMR data of these substances agreed with this result (Table 3).

Table 3. Comparison of the  $^1\text{H}$  NMR data\* for the side chain of **1**, **2**, **3** and **4**<sup>4)</sup>.

| No. | <b>4</b>     | <b>1</b>     | <b>2</b>     | <b>3</b>     |
|-----|--------------|--------------|--------------|--------------|
| 6   | 3.30 (2H, d) | 3.28 (2H, d) | 3.26 (2H, d) | 3.28 (2H, d) |
| 9   | 2.73 (2H, d) | 2.76 (2H, d) | 2.76 (2H, d) | 2.78 (2H, d) |
| 11  | 6.00 (1H, d) | 6.04 (1H, d) | 6.04 (1H, d) | 6.05 (1H, d) |
| 14  | 2.66 (1H, m) | 2.60 (1H, m) | 2.60 (1H, m) | 2.60 (1H, m) |
| 15  | 3.53 (1H, d) | 3.60 (1H, d) | 3.63 (1H, d) | 3.63 (1H, d) |
| 18  | 1.57 (3H, d) | 1.60 (3H, d) | 1.60 (3H, d) | 1.60 (3H, d) |
| 19  | 1.68 (3H, s) | 1.72 (3H, s) | 1.72 (3H, s) | 1.72 (3H, s) |
| 20  | 0.76 (3H, s) | 0.81 (3H, s) | 0.82 (3H, s) | 0.82 (3H, s) |
| 21  | 1.54 (3H, s) | 1.63 (3H, s) | 1.63 (3H, s) | 1.63 (3H, s) |
| 22  | 1.70 (3H, s) | 1.78 (3H, s) | 1.78 (3H, s) | 1.78 (3H, s) |

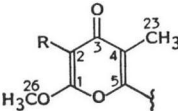
\* Measured in  $\text{CDCl}_3$  at 90 MHz with TMS as an internal standard.

Fig. 6. Structures of **7**, **8** and **9**.

When **1** was catalytically hydrogenated over Pd/C, octahydroactinopyron A ( $\text{C}_{25}\text{H}_{44}\text{O}_4$ , **5**) was obtained. The spectral data of **5**: EI-MS  $m/z$  408 ( $\text{M}^+$ );  $^1\text{H}$  NMR  $\delta$  3.90 (3H, s), 3.10 (3H, s), 2.58 (2H, t), 1.93 (3H, s), 1.82 (3H, s); UV  $\lambda_{\text{max}}^{\text{EtOH}}$  252 nm, showed that four double bonds in the side chain were hydrogenated and the remaining structure was unchanged (Fig. 5). Acetylation of **1** in acetic anhydride and pyridine gave acetate (**6**,  $\text{C}_{27}\text{H}_{38}\text{O}_5$ ).  $^1\text{H}$  NMR spectrum of **6** showed that the hydroxyl group in the side chain was acetylated<sup>3)</sup>.

The remaining fragment of **1** other than the side chain was  $\text{C}_5\text{H}_6\text{O}_3$ , which contained  $^{13}\text{C}$  signals at  $\delta$  181.0 (s), 162.1 (s), 156.8 (s), 117.8 (s), 99.3 (s), 55.1 (q), 9.8 (q), 6.8 (q),  $^1\text{H}$  signals at  $\delta$  3.86 (3H, s), 1.94 (3H, s), 1.82 (3H, s). When compared with the NMR data of other natural products, the signals of the remaining fragment were assigned to 6-substituted 2-methoxy-3,5-dimethyl-4H-pyran-4-on ring system<sup>5)</sup>. Tridathion<sup>6)</sup> (**7**), aureothin<sup>7)</sup> (**8**) and spectinabillin<sup>8)</sup> (neo-aureothin<sup>9)</sup>, **9**) are known to have this  $\gamma$ -pyrone system (Fig. 6). The comparison of  $^{13}\text{C}$  NMR data of **1**, **7**, **8** and **9** (Table 4) established the presence of this ring system as the partial structure of **1**. Thus the chemical structure of **1** was determined as depicted in Fig. 4.

Table 4. Comparison of  $^{13}\text{C}$  NMR data for the  $\alpha$ -methoxy  $\gamma$ -pyrone rings in **1**, **2**, **3**, **7** and **9**.



R =  $^{24}\text{-CH}_3$ ; **1**, **7**, **9**  
 R =  $\text{-H}$ ; **2**  
 R =  $^{24}\text{-CH}_2\text{CH}_3$ ; **3**

| No. | <b>1</b> | <b>2</b> | <b>3</b> | <b>7</b> | <b>9</b> |
|-----|----------|----------|----------|----------|----------|
| 1   | 162.1    | 167.2    | 162.2    | 161.0    | 162.1    |
| 2   | 99.3     | 88.4     | 105.3    | 97.8     | 99.1     |
| 3   | 181.0    | 181.5    | 180.4    | 181.8    | 180.6    |
| 4   | 117.8    | 118.4    | 118.4    | 118.4    | 119.9    |
| 5   | 156.8    | 159.1    | 156.9    | 160.1    | 155.2    |
| 23  | 9.8      | 9.4      | 9.8      | 11.6     | 9.4      |
| 24  | 6.8      | —        | 15.3     | 6.1      | 6.9      |
| 25  | —        | —        | 12.9     | —        | —        |
| 26  | 55.1     | 55.8     | 55.2     | 57.6     | 55.3     |

Table 5.  $^1\text{H}$  NMR data for  $\alpha$ -methoxy  $\gamma$ -pyrone rings of **1**, **2** and **3**.

| No. | <b>1</b>     | <b>2</b>     | <b>3</b>     |
|-----|--------------|--------------|--------------|
| 2   | —            | 5.45 (1H, s) | —            |
| 23  | 1.94 (3H, s) | 1.91 (3H, s) | 1.90 (3H, s) |
| 24  | 1.82 (3H, s) | —            | 2.37 (2H, q) |
| 25  | —            | —            | 1.03 (3H, t) |
| 26  | 3.86 (3H, s) | 3.80 (3H, s) | 3.89 (3H, s) |

#### Chemical Structures of **2** and **3**

The chemical structures of **2** and **3** were determined by the comparison of the  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra of **1**, **2** and **3** (Tables 2~5).

In the  $^1\text{H}$  NMR spectrum of **2**, a new signal at  $\delta$  5.45 (1H, s) was observed, and the signal at  $\delta$  1.82 (1H, s) assigned to the methyl group at C-2 carbon in **1** was not observed.  $^{13}\text{C}$ - $^1\text{H}$  selective decoupling experiment showed that the proton at  $\delta$  5.45 was attached to the carbon at  $\delta$  88.4.

Thus the structure of **2** was determined to have a hydrogen atom at C-2 carbon whereas **1** has a methyl group as depicted in Fig. 4.

In the  $^1\text{H}$  NMR spectrum of **3**, new signals at  $\delta$  2.37 (2H, q) and 1.03 (3H, t) were observed and the signal at  $\delta$  1.82 (3H, s), the methyl signal at C-2 carbon in **1**, was not observed.

Thus the structure of **3** was determined to have an ethyl group at C-2 carbon instead of the methyl group, as depicted in Fig. 4.

### Experimental

#### General

UV spectra were recorded on a Hitachi 200-20 spectrometer. IR spectra were measured on a Hitachi 285 infrared spectrometer. NMR spectra were measured on a Jeol FX 90Q spectrometer or on a Hitachi R-24B spectrometer. EI-MS were measured on a Shimadzu LKB9000B spectrometer. High resolution CI-MS were measured on a Jeol JMS-DX 300 spectrometer.

#### Reduction of **1** over 10% Pd/C

A solution of **1** (200 mg, 0.5 mm) in EtOH (50 ml) was hydrogenated in the presence of 10%

Pd/C (60 mg) for 20 hours. It was filtered and evaporated to give an oily residue. The residue was purified by silica gel column chromatography to give **5** (98 mg, 47%).

#### Acetylation of **1**

A solution of **1** (200 mg, 0.5 mm) in 1 ml of acetic anhydride and 5 ml of pyridine was stirred at room temperature for 1 hour. The product was extracted with chloroform and purified by silica gel column chromatography (benzene - EtOAc, 2: 1) to give 184 mg (86%) of acetate (**6**): IR  $\nu_{\text{max}}^{\text{film}}$   $\text{cm}^{-1}$  1735, 1665, 1595;  $^1\text{H}$  NMR (60 MHz,  $\text{CDCl}_3$ )  $\delta$  4.90 (1H, d, 15-H), 1.89 (3H, s, 15-OCOCH<sub>3</sub>); EI-MS  $m/z$  442 ( $\text{M}^+$ ,  $\text{C}_{27}\text{H}_{35}\text{O}_5$ ).

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#### References

- 1) YANO, K.; K. YOKOI, J. SATO, J. OONO, T. KOUHA, Y. OGAWA & T. NAKASHIMA: Actinopyrones A, B and C, new physiologically active substances. I. Producing organism, fermentation, isolation and biological properties. *J. Antibiotics* 39: 32~37, 1986
- 2) TAMURA, S.; N. TAKAHASHI, S. MIYAMOTO, R. MORI, S. SUZUKI & J. NAGATSU: Isolation and physiological activities of piericidin A, a natural insecticide produced by *Streptomyces*. *Agric. Biol. Chem.* 27: 576~582, 1963
- 3) TAKAHASHI, N.; A. SUZUKI & S. TAMURA: Chemical structure of piericidin A. III. Structures of piericidin A and octahydropiericidin A. *Agric. Biol. Chem.* 30: 1~12, 1966
- 4) YOSHIDA, S.; K. YONEZAWA, S. SHIRAIISHI, A. WATANABE & N. TAKAHASHI: Chemical structures of new piericidins produced by *Streptomyces pactum*. *Agric. Biol. Chem.* 41: 855~862, 1977
- 5) YAMADA, K.: Infrared and ultraviolet spectra of  $\alpha$ - and  $\gamma$ -pyrones. *Bull. Chem. Soc. Jpn.* 35: 1323~1329, 1962
- 6) IRELAND, C. & J. FAULKNER: The metabolite of marine molluscs *Tridachnella diomedea* and *Tridachna crispata*. *Tetrahedron* 37: 233~240, 1981
- 7) HIRATA, Y.; H. NAKATA, K. YAMADA, K. OKUHARA & T. NAITO: The structure of aureothin, a nitro compound obtained from *Streptomyces thioluteus*. *Tetrahedron* 14: 252~274, 1961
- 8) KAKINUMA, K.; C. A. HANSON & K. L. RINEHART, Jr.: Spectinabillin, a new nitro-containing metabolite isolated from *Streptomyces spectinabilis*. *Tetrahedron* 32: 217~222, 1976
- 9) CARDANI, C.; D. GHIRINGHELLI, A. SEVLA F. ALCAMORE, B. CAMERINO & G. CASSINELLI: Structure of neo-aureothin. *Chim. Ind. (Milan)* 52: 793~794, 1970