NAPHTHALENECARBOXYLIC ACID FROM NEOCARZINOSTATIN (NCS)

Sir:

The antitumor antibiotic neocarzinostatin (NCS), isolated from culture filtrates of Streptomyces carzinostaticus var. F-41\(^{1)}\), has been characterized as a single chain acidic protein\(^2).\) Biological studies indicate that NCS selectively inhibits DNA synthesis in sensitive cells\(^3\) in some cases and induces both DNA strand breakage\(^4\) and DNA repair synthesis\(^11)\) in other cases.

Recently, NAPIER et al. reported separation and spectral characterization of a non-protein chromophore from NCS\(^12)\). Also, IKEKI et al. independently suggested the presence of a non-protein component (NPC) separable from the protein moiety in the molecule of NCS on the basis of the results of biological studies with NCS\(^13)\). Following the last suggestion, we isolated a non-protein component and examined its biological activities\(^14)\). As a result of our continuing investigation of NCS, a naphthalene-carboxylic acid derivative (I) was obtained from NCS and NPC, and its structure was proposed on the basis of the results of some chemical transformations and spectroscopic data. In this communication we wish to report the results of the study of the structural elucidation of I along with a brief description of NPC.

A crude NPC, which was separated from NCS with methanol containing hydrochloric acid as described in the previous paper\(^14)\), was chromatographed on Sephadex LH-20. The elution pattern obtained is shown in Fig. 1. Fraction III retained as high a level of inhibitory activity against the growth of Sarcina lutea in the presence of Pre-NCS as that of NCS. After evaporation of the solvent this fraction was reduced to a brownish powder (NPC), which was found to have a chromophoric system and to be homogenous in high performance liquid chromatography (apparatus: Waters M-6,000, column: \(\mu\)-Bondapak C18 (4 mm\(\phi\) x 30 cm), eluent: formic acid-MeOH 1:9, flow rate: 0.5 ml/min at 500 psi, temperature: ambient, detector: 280 nm ± 1.0 a.u.f.s and RI, retention time: 5.6 minutes). The inhibitory activity against the growth of S. lutea was lost when NPC was exposed to light for a short time, as observed with NCS.

NPC was treated with sodium methoxide in methanol at 85°C for 3 hours, and the products were chromatographed on silica gel. Elution with n-hexane-ethyl acetate (20:1) followed by recrystallization from ethyl acetate provided colorless prisms (I), mp 104~105°C, C\(_{14}\)H\(_{14}\)O\(_{4}\). Although I showed no biological activities, this compound was found to have the same chromophore as that of NPC (UV\(_{\lambda\text{max}}\): 330 nm). The molecular formula was determined by high resolution mass spectrometry (Found: m/e 246.0886. Calcd. for C\(_{14}\)H\(_{14}\)O\(_{4}\): m/e 246.0892).

The presence of one aryl methyl group (\(\delta\) 2.63 ppm), two methoxyl groups (\(\delta\) 3.92 ppm, \(\delta\) 4.10 ppm), and four aromatic ring hydrogens in the molecule of I was indicated by the nuclear magnetic resonance (NMR) spectrum (Fig. 2). The presence of an associated hydroxyl group in the structure of I was suggested by the broad absorption band centered at 3350 cm\(^{-1}\) in the infrared absorption (IR) spectrum (CCl\(_{3}\)). After treatment with diazomethane for an extended Fig. 1. Chromatography of crude NPC on a Sephadex LH-20 column.

Crude NPC separated from NCS (30 g) was applied to a Sephadex LH-20 column (3.1 x 138 cm) and eluted with 1 N HCl-MeOH (1:9). The optical densities at 280 nm (—) and 330 nm (—) indicated four peaks. Respective yields were Fraction I, 4336 mg; Fraction II, 329 mg; Fraction III, 263 mg; Fraction IV, 11 mg by dry weight. Inhibitory activity against the growth of S. lutea in the presence of Pre-NCS was found in the cross-hatched area.
time I provided a methyl ether (II) as colorless prisms, mp 151~151.5°C, C_{15}H_{16}O_{4}. Compounds I and II had Rf values of 0.8 and 0.3 respectively on the TLC system with n-hexane - ethyl acetate (10:1). Similar behavior on TLC was observed with peanol, salicylic acid and 5-hydroxyflavonoids and their methyl esters. Furthermore, I was not readily acetylated. However an acetyl derivative (III), mp 166~167°C, C_{16}H_{16}O_{5}, m/e 288 in the mass spectrum (MS), was obtained by heating I with acetic anhydride and anhydrous sodium sulfate. Gas chromatography-mass spectrometry of I after exchange with D_{2}O afforded the parent ion at m/e 247 (one exchangeable proton). Thus, the presence of a strongly associated hydroxyl group in the molecule of I was certified from the above mentioned results.

The fact that one of the two methoxyl groups in I is associated with a methoxycarbonyl group was based on the result of the hydrolysis of II. On treatment with 5% NaOH (H_{2}O - MeOH - dioxane, 1:1:1) II provided a free carboxylic acid (IV), mp 133~134°C, C_{14}H_{14}O_{4}, which showed Rf 0.2 on TLC with n-hexane - ethyl acetate (1:1), while I and II showed spots at the solvent front. Therefore, a methoxycarbonyl group was confirmed in the structure of I, an assignment supported by a band at 1725 cm^{-1} in the IR spectrum (CCl_{4}) (Table 1, Fig. 3) of I. So far in this analysis, an aryl methyl, a methoxyl, a methoxycarbonyl, a hydroxyl group and four aromatic ring hydrogens, account for C_{4}H_{14}O_{4} at the molecular formula of I, leaving ten carbons to be clarified. Thus, a naphthalene skelton was proposed for the ten carbons and was supported by the results of the ultraviolet (UV) absorption maximum at 330 nm (Fig. 4).

The positions of the aryl methyl, methoxyl, methoxycarbonyl and hydroxyl groups and four aromatic ring hydrogens on a naphthalene skelton were determined on the basis of spectroscopic data.

Table 1. Physical and spectroscopic data of I~IV.

<table>
<thead>
<tr>
<th></th>
<th>R_{1}</th>
<th>R_{2}</th>
<th>mp (°C)</th>
<th>MS (m/e)</th>
<th>IR (cm^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>CH_{3}</td>
<td>H</td>
<td>104~105</td>
<td>M^+ 246.0886 (C_{14}H_{12}O_{4}), F^+ 186, 161</td>
<td>3350, 2970, 2940, 2870, 1725, 1655, 1645, 1610, 1200 (CCl_{4})</td>
</tr>
<tr>
<td>II</td>
<td>CH_{3}</td>
<td>CH_{3}</td>
<td>151~151.5</td>
<td>M^+, 260 (C_{13}H_{10}O_{3}), F^+, 229 (M^+-OCH_{3})</td>
<td>3020, 2950, 2860, 1720, 1622, 1270 (CHCl_{3})</td>
</tr>
<tr>
<td>III</td>
<td>CH_{3}</td>
<td>COCH_{3}</td>
<td>166~167</td>
<td>M^+, 288 (C_{13}H_{10}O_{3}), F^+, 256, 246, 214, 167, 149</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>H</td>
<td>CH_{3}</td>
<td>133~134</td>
<td>M^+, 246 (C_{14}H_{12}O_{4}), F^+, 229 (M^+-OH)</td>
<td>3500~3000, 3020, 2950, 2860, 1720, 1705, 1622, 1265 (CHCl_{3})</td>
</tr>
</tbody>
</table>

\*1 R_{1}, R_{2}: ones of Chart 2 \\
\*2 M^+: molecular ion \\
\*3 F^+: fragment ion(s).
In the NMR spectrum of I, a pair of doublets (δ 8.01 ppm and 7.01 ppm, J=9.3 Hz) was attributed to ortho coupled hydrogens. The doublet in the lower field was attributed to a hydrogen under paramagnetic effect of the methoxycarbonyl group. The fact that the aryl methyl group was flanked by two aromatic ring hydrogens was shown by NMR decoupling experiments. Upon irradiation at δ 2.63 ppm (aryl methyl), an unresolved signal at δ 6.90 ppm was found to collapse into a doublet of J=2.5 Hz, although any conversion of a signal of another aromatic ring hydrogen was not recognized because of overlapping with the above mentioned doublet at the lower field. Reversely, upon irradiation at δ 6.90 ppm or 8.01 ppm, the broad singlet of the aryl methyl group at δ 2.63 ppm, was found to collapse into a doublet-like signal. A nuclear OVERHAUSER effect (18%) was found between the methoxyl group and the paramagnetically shifted member of the ortho coupled hydrogens. The fragmentation pattern of I in MS was very similar to that of methyl salicylate, particularly when in light

| Table 2. NMR spectral data of I, II and IV. (δ, ppm) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | solvent         | R<sub>1</sub> | R<sub>2</sub> | C<sub>3</sub>-H | C<sub>3</sub>-H | C<sub>5</sub>-OCH<sub>3</sub> | C<sub>5</sub>-H | C<sub>7</sub>-CH<sub>3</sub> | C<sub>5</sub>-H<sup>2</sup> |
| I<sup>4</sup>  | CDCl<sub>3</sub> | R<sub>1</sub>=CH<sub>3</sub> 4.10 | — | 7.01 | 8.01 | 3.92 | 6.90 | 2.63 | 8.09 |
|                |                 | R<sub>2</sub>=H | — | d, J=9.3 | d, J=9.3 | s<sup>85</sup> | u<sup>85</sup> | s | u |
| II<sup>4</sup> | CCl<sub>4</sub> | R<sub>1</sub>=CH<sub>3</sub> 3.89 | — | 6.91 | 7.77 | 3.82 | 6.73 | 2.57 | 6.73 |
|                |                 | R<sub>2</sub>=CH<sub>3</sub> 3.65 | d, J=9.0 | d, J=9.0 | s | u | s | u |
| IV<sup>4</sup> | CDCl<sub>3</sub> | R<sub>1</sub>=H | — | 7.12 | 8.02 | 3.90 | 6.90 | 2.63 | 7.74 |
|                |                 | R<sub>2</sub>=CH<sub>3</sub> 4.06 | (3.90) | d, J=9.0 | d, J=9.0 | (4.06) | u | s | u |

<sup>*1</sup> R<sub>1</sub>, R<sub>2</sub>: ones of Chart 2. <sup>*2</sup> numbering: that of Chart 2. <sup>*3</sup>: doublet, s: singlet, u: unresolved.
<sup>*4</sup> Data of I were determined in FX-100 of JEOC, while data of II and IV were determined in EM-390 90 Hz NMR spectrometer.
of the ortho effect described as a McLafferty rearrangement. On the basis of these results, methyl 2-hydroxy-5-methoxy-7-methyl-1-naphthalencarboxylate was proposed for the chemical structure of I. This proposal was supported by the results of a detailed comparison of NMR spectral data of I, II and IV as summarized in Table 2.

NPC provided compound V and VI on treatment with sodium methoxide d-3 in methanol d-4 or sodium ethoxide in ethanol, respectively. V and VI showed the parent peaks at m/e 249 (C_{14}H_{11}D_{3}O_{4}) and m/e 260 (C_{15}H_{16}O_{4}) in MS (Chart 1). These facts indicated that deuterated methanol or ethanol was incorporated into the carboxylate group. Therefore, a structural transformation must take place in the moiety of the carbonyl group at C_1 of a naphthalene derivative in the course of the preparation of I from NPC. The latter retains the biological active site of NCS and is characterized by a high sensitivity to light and by possessing a specific IR band at 1780 cm^{-1}. Studies directed towards the structural elucidation of this substance are progressing.

A similar naphthalencarboxylic acid derivative, 3-methoxy-5-methyl-1-naphthalencarboxylic acid (VII), has been isolated from the antitumor antibiotic carzinophilin (CP). The role of these naphthalencarboxylic acids of NCS and CP has not been clarified yet, but they certainly have some important relationship to the biological activities of these antibiotics. Other antitumor antibiotics, such as auromycin which has been shown to have a chromophore, might contain similar naphthalencarboxylic acid derivatives.

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