# TAN-931, A NOVEL NONSTEROIDAL AROMATASE INHIBITOR PRODUCED BY *Penicillium funiculosum* No. 8974

## II. STRUCTURE ELUCIDATION, CHEMICAL MODIFICATION AND BIOLOGICAL ACTIVITY

TSUNEAKI HIDA, TAKAFUMI ISHII, TSUNEO KANAMARU and MASAYUKI MUROI

Microbiology Research Laboratories, Research and Development Division,
Takeda Chemical Industries, Ltd.,
2-17-85 Jusohonmachi, Yodogawa-ku, Osaka 532, Japan

(Received for publication January 16, 1991)

The structure of TAN-931, a novel nonsteroidal aromatase inhibitor, was determined by chemical reactions and spectral analyses including 2D NMR experiments to be 4-(2,6-dihydroxybenzoyl)-3-formyl-5-hydroxybenzoic acid. Several derivatives of TAN-931 were prepared, and it was found that the 3-formyl and 2'- and/or 6'-hydroxyl groups play an important role in its inhibitory activity. Among the compounds synthesized, 4-(2,6-dihydroxybenzoyl)-3-formyl-5-methoxy-*N*,*N*-dimethylbenzamide was found to be more effective than TAN-931 when administered orally.

In the course of our screening program in search of new aromatase inhibitors of microbial origin, TAN-931 (**1**) was discovered in the culture filtrate of *Penicillium funiculosum* No. 8974. In the previous paper[1], we reported the taxonomy of the producing organism and the fermentation, isolation, characterization and biological activities of this inhibitor. We describe here the structure elucidation and chemical modification of **1** and biological activity of derivatives.

### Structure Elucidation

The IR spectrum of **1**[1] had absorption bands at 3600~2500 (br) and 1720 $cm^{-1}$, indicating the presence of a carboxyl group, which was supported by formation of the methyl ester (**2**) by brief treatment of **1** with diazomethane. The UV spectrum of **1**[1] in MeOH had maxima at 223, 275 and 336 nm. In alkaline solution, these shifted to 235 (sh), 275 (sh) and 385 nm, respectively.

In the $^1H$ NMR spectrum of **1** (Table 1), a singlet signal at $\delta$ 9.94 suggested the presence of an aldehyde. Doublets at $\delta$ 8.01 and 7.72 were coupled ($J$=1.2 Hz), and another doublet at $\delta$ 6.29 (2H, $J$=8.2 Hz) was coupled with a triplet at $\delta$ 7.28. Phenol protons were observed at $\delta$ 10.42 (1H) and 11.44 (2H). The presence of three phenol groups was further confirmed by permethylation with dimethyl sulfate-$K_2CO_3$. The tetramethyl derivative (**3**) thus obtained contains three methoxyl groups and one methyl ester group. In the $^{13}C$ NMR spectrum of **1** (Table 1), carbonyl signals at $\delta$ 200.0, 192.0 and 166.1 were assigned to a ketone, an aldehyde and a carboxyl group, respectively. The signals at $\delta$ 106.9 and 161.8 were duplicated, and resonances for two carbons were assumed to overlap at $\delta$ 134.2. These findings suggested that **1** has two aromatic rings connected by a ketone like benzophenone; one of the rings (Ring A) bears an aldehyde, a carboxylic acid and a hydroxyl group, and the other ring (Ring B) has a 2',6'-dihydroxy-substituent. Two meta-

Fig. 1. Structure of TAN-931 (**1**).

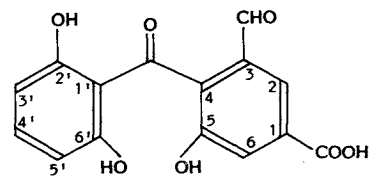


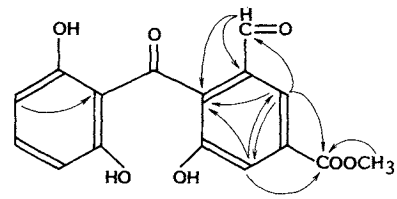
Table 1. NMR chemical shifts ($\delta$ ppm) of TAN-931 (**1**) and **2**.

| | | TAN-931 (**1**) | | **2** | |
|---|---|---|---|---|---|
| | | $^{13}C$ | $^{1}H$ ($J$=Hz) | $^{13}C$ | $^{1}H$ ($J$=Hz) |
| Ring A | 1 | 131.8 | | 130.5 | |
| | 2 | 124.1 | 8.01 d (1.2) | 123.8 | 8.04 d (1.4) |
| | 3 | 134.2 | | 134.2 | |
| | 4 | 134.2 | | 134.5 | |
| | 5 | 153.7 | | 153.7 | |
| | 6 | 121.7 | 7.72 d (1.2) | 121.3 | 7.74 d (1.4) |
| Ring B | 1′ | 110.8 | | 110.7 | |
| | 2′ (6′) | 161.8 | | 161.7 | |
| | 3′ (5′) | 106.9 | 6.29 d (2H, 8.2) | 106.9 | 6.30 d (2H, 8.2) |
| | 4′ | 137.0 | 7.28 t (8.2) | 137.0 | 7.28 t (8.2) |
| Carbonyl | | 200.0 | | 199.7 | |
| Carboxyl | | 166.1 | | 165.1 | |
| Formyl | | 192.0 | 9.94 s | 191.9 | 9.95 s |
| 5-OH | | | 10.42 br | | 10.50 br |
| 2′,6′-OH | | | 11.44 br (2H) | | 11.45 br (2H) |
| COOH | | | 13.31 br | | |
| $OCH_3$ | | | | 52.4 | 3.91 s |

coupled protons on Ring A were observed at $\delta$ 7.72 and 8.01 ($J$=1.2 Hz).

Upon treatment of **1** with trifluoroacetic acid (TFA) in MeOH, a methylacetal (**4**) was obtained. In the $^{1}H$ NMR spectrum of **4**, the aldehyde proton disappeared and an acetal signal ($\delta$ 6.19) together with a methoxyl signal ($\delta$ 3.46) appeared. This compound is assumed to be formed through a neighboring-group interaction between a formyl- and either 2′- or 6′-hydroxy-groups of the substituted benzophenone as in the case of arugosin[2].

Fig. 2. COLOC experiment of **2**.
$^{1}H \rightarrow ^{13}C$

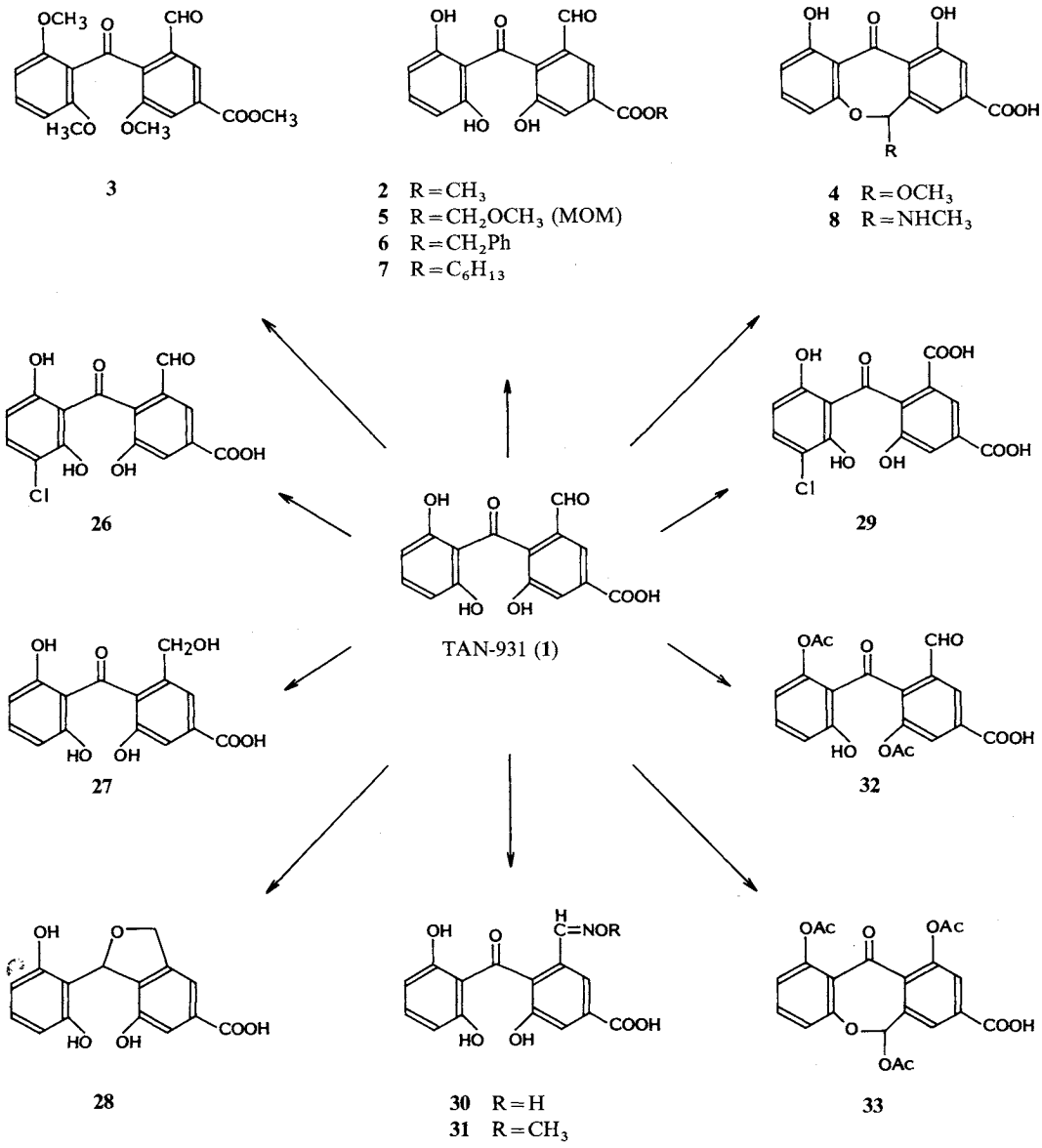



2D NMR techniques ($^{13}C$,$^{1}H$-COSY[3] and correlation *via* long range coupling (COLOC)[4]) were applied to the methyl ester (**2**) in which the six carbon signals on Ring A were separated and easy to analyze. Connectivities from NMR experiments with COLOC linkages are shown in Fig. 2. One of the two protons on Ring A (2-H; $\delta$ 8.04) coupled to the aldehyde carbon and the carboxyl carbon, and the other proton (6-H; $\delta$ 7.74) coupled to the carboxyl carbon. These findings indicated that Ring A has 1-carboxyl, 3-formyl and 5-hydroxyl groups as substituents and is connected to Ring B by the ketone at the C-4 position. Therefore, the structure of **1** was concluded to be 4-(2,6-dihydroxybenzoyl)-3-formyl-5-hydroxybenzoic acid (Fig. 1).

## Chemical Modification of **1**

Chemical modification of **1** is outlined in Schemes 1, 2 and 3. Besides the methyl ester (**2**), other ester derivatives (**5**~**7**) were synthesized by reaction with alkyl halides in the presence of sodium hydrogen carbonate. Although direct reaction of **1** with methylamine easily afforded the aminoacetal (**8**) *via* cyclization of the formyl and hydroxyl groups, reaction of the methylacetal (**4**) with amines in the presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) and subsequent acid hydrolysis

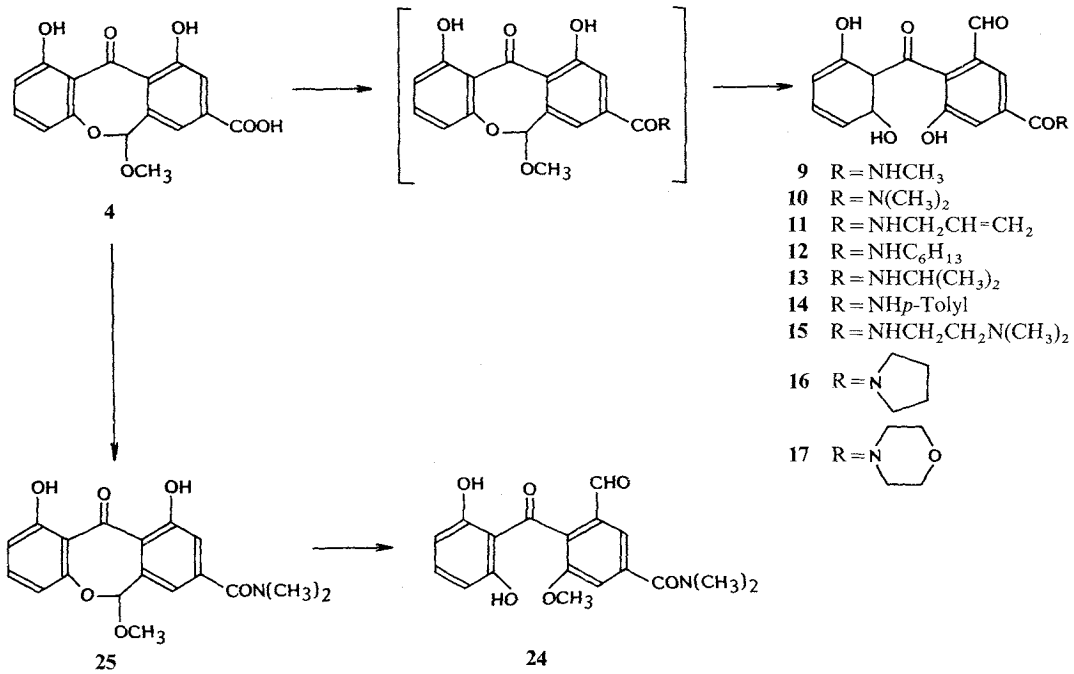
Scheme 1.



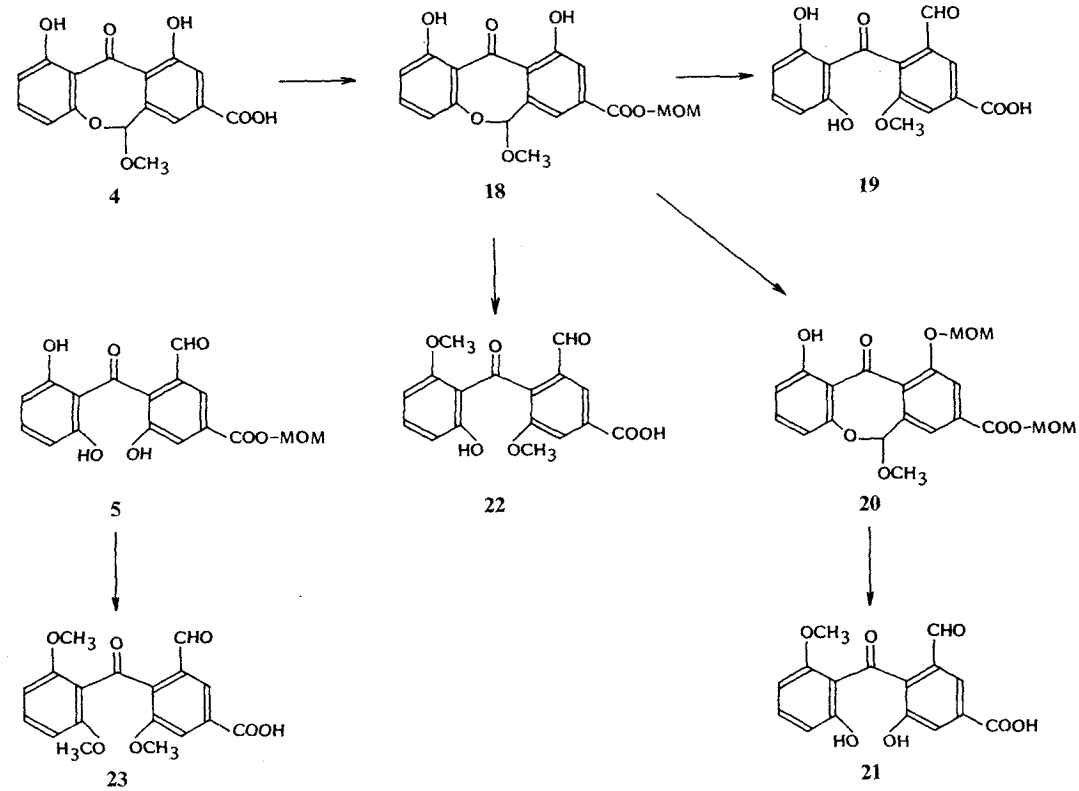

gave a series of amide derivatives (**9**~**17**).

For the selective methylation of phenol groups, the carboxyl group of methylacetal **4** was protected as a methoxymethyl (MOM) ester (**18**). Monomethylation of **18** was carried out with dimethyl sulfate (1 equiv) and $K_2CO_3$, and the compound thus obtained was hydrolyzed to give monomethyl ether **19**. The position of the methoxyl group should therefore be at C-5 not at C-2', since in the $^1H$ NMR spectrum the 3'-H and 5'-H protons in **19** were identical. The C-5 phenol group in **18** was protected as a MOM ether (**20**), and subsequent methylation with dimethyl sulfate and deprotection with acid yielded the 2'-*O*-methyl derivative (**21**) whose 3'-H and 5'-H proton signals were observed individually in the $^1H$ NMR spectrum. Excess dimethyl sulfate with **18** and **5** followed by acid hydrolysis gave the 2',5-di-*O*-methyl

Scheme 2.




Scheme 3.



derivative (**22**) and the 2′,5,6′-tri-*O*-methyl derivative (**23**), respectively. Compound **24** was prepared by selective methylation of **25**, which is the *N*,*N*-dimethylamide derivative of the methylacetal (**4**), and subsequent acid hydrolysis. Chlorination of **1** was carried out with *N*-chlorosuccinimide to give **26**.

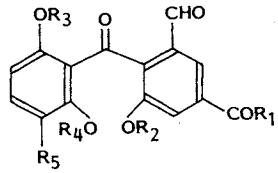
Hydrogenation of **1** over Pd-C gave the hydroxymethyl derivative (**27**), and a prolonged reaction time led to the phthalan derivative (**28**)[2]. Oxidation of **1** with sodium chlorite in the presence of sulfamic acid[5] gave the diacid (**29**) which has a chlorine atom at the C-3′ position. The aldehyde group of **1** was easily converted to oximes (**30** and **31**).

Acetylation of **1** with $Ac_2O$ in pyridine gave the diacetyl derivative (**32**), however, the reaction in the presence of 4-dimethylaminopyridine (DMAP) led to the cyclic triacetate (**33**)[6].

## Biological Activity and Discussion

In Table 2, the $IC_{50}$ values of the derivatives against aromatase from human placenta are shown. Although the methyl and methoxymethyl esters (**2** and **5**) were as effective as **1**, esterification at the C-1 position with hydrophobic groups (**6** and **7**) tended to result in diminished activity. Conversion of the carboxyl group to amides (**9**～**17**) did not cause a marked change of activity even when there were hydrophobic alkyl or aryl groups on the nitrogen atom.

Table 2. Inhibitory activity of derivatives.




| Compound | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $IC_{50}$ ($\mu$M) |
|---|---|---|---|---|---|---|
| **1** | OH | H | H | H | H | 17 |
| **2** | $OCH_3$ | H | H | H | H | 22 |
| **5** | $OCH_2OCH_3$ | H | H | H | H | 12 |
| **6** | $OCH_2Ph$ | H | H | H | H | 207 |
| **7** | $OC_6H_{13}$ | H | H | H | H | 34 |
| **9** | $NHCH_3$ | H | H | H | H | 21 |
| **10** | $N(CH_3)_2$ | H | H | H | H | 15 |
| **11** | $NHCH_2CH{=}CH_2$ | H | H | H | H | 15 |
| **12** | $NHC_6H_{13}$ | H | H | H | H | 18 |
| **13** | $NHCH(CH_3)_2$ | H | H | H | H | 26 |
| **14** | NH*p*-Tolyl | H | H | H | H | 16 |
| **15** | $NHCH_2CH_2N(CH_3)_2$ | H | H | H | H | 15 |
| **16** |  | H | H | H | H | 23 |
| **17** |  | H | H | H | H | 16 |
| **19** | OH | $CH_3$ | H | H | H | 24 |
| **21** | OH | H | $CH_3$ | H | H | 136 |
| **22** | OH | $CH_3$ | $CH_3$ | H | H | 97 |
| **23** | OH | $CH_3$ | $CH_3$ | $CH_3$ | H | >500 |
| **3** | $OCH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | H | >500 |
| **24** | $N(CH_3)_2$ | $CH_3$ | H | H | H | 18 |
| **26** | OH | H | H | H | Cl | 14 |
| **32** | OH | Ac | Ac | H | H | 16 |

Table 3. Effect of TAN-931 (**1**) on the weight of the uterus and ovaries and the plasma estradiol-17$\beta$ level in rats.

| Compound | Dose (mg/kg) | Gain of body weight (g) | Weight of | | Plasma $E_2$[a] (pg/ml) |
|---|---|---|---|---|---|
| | | | Uterus (mg) | Ovaries (mg) | |
| None | — | 21.8±1.1 | 27.8± 2.6 | 15.9±3.0 | 3.3± 4.3 |
| PMSG treatment | — | 19.8±1.6 | 106.7±20.2 | 29.5±2.3 | 224.9±35.9 |
| **1** (po) | 100 | 19.0±1.9 | 100.6±10.1 | 35.0±4.3 | 246.0±71.7 |

Mean±SD, n=6.
PMSG: Pregnant more serum gonadotropin.
[a] Plasma estradiol-17$\beta$ level.

Table 4. Effect of TAN-931 (**1**) and **24** on the weight of the uterus and ovaries, the plasma estradiol-17$\beta$ level, and ovarian aromatase activity in rats.

| Compound | Dose (mg/kg) | Gain of body weight (g) | Weight of | | Plasma $E_2$ (pg/ml) | Ovarian aromatase | |
|---|---|---|---|---|---|---|---|
| | | | Uterus (mg) | Ovary (mg) | | Total (U/ovary) | Specific (U/mg) |
| None | — | 12.2±1.6 | 32.5± 8.8 | 18.4±3.0 | 55.4± 7.3 | 3.1 | 62.4 |
| PMSG treatment | — | 12.2±1.3 | 132.6±11.4 | 38.5±9.4 | 331.9±185.7 | 18.3 | 131.2 |
| **1** (sc) | 100 | 13.7±1.9 | 50.6±32.3** | 17.8±2.9** | 66.7± 12.0** | 4.7 | 65.2 |
| **24** (po) | 100 | 10.5±1.4 | 115.1± 4.9* | 32.4±7.0 | 216.7± 73.0 | 12.5 | 109.5 |

Mean±SD, n=6.
Student's t-test against PMSG-group, * $P<0.01$, ** $P<0.001$.
One U of aromatase activity was defined as fmol $^3H_2O$ formed/minute.
Specific activity was defined as units/mg ovarian microsomal protein.

Conversion of the aldehyde group to hydroxymethyl (**27**), carboxyl (**29**) and oxime (**30** and **31**) groups caused a complete loss of activity. Neither the methylacetal (**4**) nor the aminoacetal (**8**) showed any activity.

Introduction of a methyl group onto the C-5 phenol group had little influence on activity, however, methylation of the C-2′ phenol group resulted in reduced activity, and methylation of both the C-2′ and C-6′ phenol groups caused a loss of activity. These findings revealed that the 3-formyl and 2′- and/or 6′-hydroxyl groups play an essential role in aromatase inhibition.

Although in *in vivo* experiments **1** had no effect upon oral administration at a dose of 100 mg/kg (Table 3), the *N,N*-dimethylamide derivative (**24**) was more effective (Table 4). When **24** was orally administered at a dose of 100 mg/kg, the weight of the uterus was significantly reduced. Moreover, the plasma estradiol-17$\beta$ level and ovarian aromatase activity were also reduced. These findings suggest that changing the carboxyl group to an amide group has the potential to increase the bioavailability of **1**.

The currently known nonsteroidal aromatase inhibitors are aminoglutethimide[7~9], naphthoflavone derivatives[10,11], and imidazole derivatives[12,13], however, **1** does not belong to any of these three categories and therefore is a novel type of nonsteroidal aromatase inhibitor.

## Experimental

### General

MP's were uncorrected. UV spectra were taken on a Hitachi 320 spectrophotometer. IR spectra were obtained with a Hitachi 285 grating IR spectrophotometer using a KBr disk. NMR spectra were recorded on Bruker AC-300 instrument ($^1H$, 300 MHz; $^{13}C$, 75 MHz): Chemical shifts ($\delta$) are reported in ppm downfield from internal TMS in DMSO-$d_6$ solutions; coupling constants are reported in Hz. Merck Silica

gel 60 was used for column chromatography.

Methyl Ester (**2**)

Ethereal diazomethane was added to a solution of **1** (50 mg, 0.17 mmol) in THF (2 ml), and the solution was allowed to stand for 10 minutes at room temperature. The reaction mixture was concentrated, and the resulting residue was chromatographed on Sephadex LH-20 eluting with MeOH. The pure fraction was concentrated and crystallized from EtOAc-hexane to give **2** as yellow crystals (40 mg, 76%): MP 173.5~175°C; IR $\nu_{max}$ $cm^{-1}$ 1720, 1630, 1595.

*Anal* Calcd for $C_{16}H_{12}O_7$: C 60.76, H 3.82.
Found: C 60.72, H 3.78.

Tetramethyl Derivative (**3**)

Anhydrous $K_2CO_3$ (500 mg, 3.6 mmol) and dimethyl sulfate (0.5 ml, 5.3 mmol) were added to a suspension of **1** (100 mg, 0.33 mmol) in $Me_2CO$ (2 ml). The reaction mixture was refluxed for 2 hours with stirring, and the solid residue was removed by filtration. The filtrate was concentrated and the residue was triturated with ethyl ether to give a powder. The powder was dissolved in EtOAc (20 ml), washed with water and concentrated. The residue was crystallized from EtOAc to give **3** as pale yellow crystals (91 mg, 77%): MP 166~167°C; $^1H$ NMR $\delta$ 3.62 (6H, s), 3.71 (3H, s), 3.91 (3H, s), 6.71 (2H, d, $J$=8.4 Hz), 7.42 (1H, t, $J$=8.4 Hz), 7.76 (1H, d, $J$=1.4 Hz), 8.00 (1H, d, $J$=1.4 Hz), 9.96 (1H, s); IR $\nu_{max}$ $cm^{-1}$ 1730, 1690, 1600.

*Anal* Calcd for $C_{19}H_{18}O_7$: C 63.68, H 5.06.
Found: C 63.80, H 5.06.

Methylacetal (**4**)

TFA (0.1 ml, 1.3 mmol) was added to a solution of **1** (50 mg, 0.17 mmol) in MeOH (5 ml), and the solution was stirred for 30 minutes at room temperature. The reaction mixture was concentrated to give crude crystals. Recrystallization from MeOH gave yellow crystals of **4** (42 mg, 80%): MP 268~271°C (dec); $^1H$ NMR $\delta$ 3.46 (3H, s), 6.19 (1H, s), 6.63 (1H, dd, $J$=8.2 and 1.0 Hz), 6.68 (1H, dd, $J$=8.2 and 1.0 Hz), 7.50 (1H, t, $J$=8.2 Hz), 7.57 (1H, d, $J$=1.4 Hz), 7.61 (1H, d, $J$=1.4 Hz), 10.59 (1H, br), 12.12 (1H, br s); IR $\nu_{max}$ $cm^{-1}$ 1720, 1630, 1595.

*Anal* Calcd for $C_{16}H_{12}O_7$: C 60.76, H 3.82.
Found: C 60.76, H 3.77.

Methoxymethyl Ester (**5**)

$NaHCO_3$ (1.12 g, 13 mmol) and methoxymethyl chloride (380 $\mu$l, 5.0 mmol) were added to a solution of **1** (1.00 g, 3.3 mmol) in DMF (10 ml). The mixture was stirred at room temperature for 1 hour and diluted with EtOAc (50 ml). The mixture was washed with 1 N hydrochloric acid, water and brine (20 ml). The organic layer was dried and the solvent was removed. The residue was chromatographed on silica gel, eluting with $CHCl_3$-MeOH (20:1), to give 810 mg (73%) of **5** as yellow crystals (from EtOAc-hexane): MP 143~145°C; $^1H$ NMR $\delta$ 3.49 (3H, s), 5.50 (2H, s), 6.30 (2H, d, $J$=8.2 Hz), 7.28 (1H, t, $J$=8.2 Hz), 7.78 (1H, d, $J$=1.4 Hz), 8.08 (1H, d, $J$=1.4 Hz), 9.97 (1H, s), 10.52 (1H, br s), 11.44 (2H, br s); IR $\nu_{max}$ $cm^{-1}$ 1730, 1630.

*Anal* Calcd for $C_{17}H_{14}O_8$: C 58.96, H 4.07.
Found: C 59.02, H 4.08.

In a similar manner using benzyl bromide and *n*-hexyl iodide, compounds **6** and **7**, respectively, were prepared from **1**.

**6**: Yield 83%; yellow crystals (from EtOAc-hexane); mp 184.5~186°C; $^1H$ NMR $\delta$ 5.41 (2H, s), 6.29 (2H, d, $J$=8.2 Hz), 7.28 (1H, t, $J$=8.2 Hz), 7.35~7.55 (5H, m), 7.78 (1H, d, $J$=1.4 Hz), 8.07 (1H, d, $J$=1.4 Hz), 9.96 (1H, s), 10.51 (1H, br), 11.45 (2H, br); IR $\nu_{max}$ $cm^{-1}$ 1730, 1630.

*Anal* Calcd for $C_{22}H_{16}O_7$: C 67.35, H 4.11.
Found: C 67.15, H 4.29.

**7**: Yield 69%; yellow crystals (from EtOAc-hexane); mp 129.5~130°C; $^1H$ NMR $\delta$ 0.89 (3H, br t, $J$=7.0 Hz), 1.2~1.5 (6H, m), 1.74 (2H, m), 4.32 (2H, t, $J$=6.6 Hz), 6.30 (2H, d, $J$=8.2 Hz), 7.28 (1H, t, $J$=8.2 Hz), 7.75 (1H, d, $J$=1.4 Hz), 8.02 (1H, d, $J$=1.4 Hz), 9.95 (1H, s), 10.47 (1H, br), 11.43 (2H, br);

IR $\nu_{max}$ $cm^{-1}$ 1730, 1625.

*Anal* Calcd for $C_{21}H_{22}O_7$: C 65.28, H 5.74.
Found: C 64.98, H 6.05.

Compound **18** was prepared in a similar manner using **4** and methoxymethyl chloride.

**18**: Yield 77%; yellow crystals (from EtOAc-hexane); mp 135.5~136°C; $^1$H NMR $\delta$ 3.47 (3H, s), 3.48 (3H, s), 5.47 (2H, s), 6.22 (1H, s), 6.63 (1H, dd, $J$=8.2 and 1.1 Hz), 6.68 (1H, dd, $J$=8.2 and 1.1 Hz), 7.50 (1H, t, $J$=8.2 Hz), 7.63 (1H, d, $J$=1.6 Hz), 7.66 (1H, d, $J$=1.6 Hz), 10.65 (1H, br), 12.03 (1H, br s); IR $\nu_{max}$ $cm^{-1}$ 1735, 1625.

*Anal* Calcd for $C_{18}H_{16}O_8$: C 60.00, H 4.48.
Found: C 60.30, H 4.58.

Aminoacetal (**8**)

Methylamine hydrochloride (24.6 mg, 0.36 mmol) and triethylamine (TEA, 49 $\mu$l, 0.35 mmol) were added to a solution of **1** (100 mg, 0.33 mmol) in DMF (1.0 ml). The mixture was stirred at room temperature for 15 minutes, poured into water (20 ml) and extracted with EtOAc (3 × 30 ml) at pH 3.0. The organic layers were combined and washed with water and brine. After concentration, the residue was crystallized from EtOAc to give **8** as colorless crystals (89 mg, 85%): MP 100°C (dec); $^1$H NMR $\delta$ 2.74 (3H, s), 6.03 (1H, s), 6.05 (1H, br d, $J$=8.1 Hz), 6.39 (1H, br d, $J$=8.1 Hz), 6.87 (1H, t, $J$=8.1 Hz), 7.46 (1H, d, $J$=1.3 Hz), 7.63 (1H, d, $J$=1.3 Hz), 8.79 (1H, s), 9.74 (1H, br); IR $\nu_{max}$ $cm^{-1}$ 1695, 1650, 1600.

*Anal* Calcd for $C_{16}H_{13}NO_6 \cdot \frac{1}{2}H_2O$: C 59.26, H 4.35, N 4.32.
Found: C 59.18, H 4.41, N 4.34.

Methylamide (**9**)

Methylamine hydrochloride (93 mg, 1.4 mmol), TEA (195 $\mu$l, 1.4 mmol), HOBT (187 mg, 1.4 mmol) and DCC (284 mg, 1.4 mmol) were added to a solution of **4** (400 mg, 1.3 mmol) in DMF (4.0 ml). The reaction mixture was stirred at room temperature for 2 hours and then diluted with EtOAc (60 ml). The mixture was filtered, and the filtrate was washed successively with 2% aq $NaHCO_3$, 1 N hydrochloric acid, water and brine. The organic layer was concentrated and the crystalline ressidue was dissolved in THF (8 ml) and 1 N hydrochloric acid (2 ml). The mixture was stirred at 50°C for 16 hours, diluted with EtOAc (100 ml), and washed with water and brine. The organic solution thus obtained was dried and concentrated. The residue was crystallized from $CHCl_3$-MeOH to give **9** (193 mg, 48%) as yellow crystals: MP 222~226°C (dec); $^1$H NMR $\delta$ 2.81 (3H, d, $J$=4.5 Hz), 6.29 (2H, d, $J$=8.2 Hz), 7.27 (1H, t, $J$=8.2 Hz), 7.60 (1H, d, $J$=1.4 Hz), 7.89 (1H, d, $J$=1.4 Hz), 8.63 (1H, q, $J$=4.5 Hz), 9.89 (1H, s), 10.27 (1H, br), 11.44 (2H, br); IR $\nu_{max}$ $cm^{-1}$ 1625.

*Anal* Calcd for $C_{16}H_{13}NO_6$: C 60.95, H 4.16, N 4.44.
Found: C 60.78, H 4.17, N 4.44.

Compounds **10**~**17** were prepared from **4** in a manner similar to that used for the preparation of **9**.

**10**: Yield 75%; yellow crystals (from EtOAc-hexane); mp 198~200°C; $^1$H NMR $\delta$ 2.97 (3H, br s), 3.01 (3H, br s), 6.29 (2H, d, $J$=8.2 Hz), 7.15 (1H, d, $J$=1.3 Hz), 7.26 (1H, t, $J$=8.2 Hz), 7.47 (1H, d, $J$=1.3 Hz), 9.87 (1H, s), 10.30 (1H, br), 11.47 (2H, br); IR $\nu_{max}$ $cm^{-1}$ 1685, 1625.

*Anal* Calcd for $C_{17}H_{15}NO_6$: C 62.00, H 4.59, N 4.25.
Found: C 62.12, H 4.81, N 4.40.

**11**: Yield 64%; yellow crystals (from EtOAc-hexane); mp 173~174°C (dec); $^1$H NMR $\delta$ 3.93 (2H, m), 5.12 (1H, dq, $J$=10.2 and 1.6 Hz), 5.19 (1H, dq, $J$=17.2 and 1.6 Hz), 5.91 (1H, ddt, $J$=17.2, 10.2 and 5.1 Hz), 6.29 (2H, d, $J$=8.2 Hz), 7.27 (1H, t, $J$=8.2 Hz), 7.63 (1H, d, $J$=1.4 Hz), 7.95 (1H, d, $J$=1.4 Hz), 8.86 (1H, t, $J$=5.8 Hz), 9.89 (1H, s), 10.30 (1H, br), 11.44 (2H, br); IR $\nu_{max}$ $cm^{-1}$ 1625.

*Anal* Calcd for $C_{18}H_{15}NO_6$: C 63.34, H 4.43, N 4.10.
Found: C 63.36, H 4.56, N 4.18.

**12**: Yield 64%; pale yellow crystals (from $CHCl_3$); mp 217~221°C (dec); $^1$H NMR $\delta$ 0.88 (3H, br t, $J$=6.7 Hz), 1.30 (6H, m), 1.54 (2H, m), 3.27 (2H, m), 6.29 (2H, d, $J$=8.2 Hz), 7.27 (1H, t, $J$=8.2 Hz), 7.60 (1H, d, $J$=1.3 Hz), 7.90 (1H, d, $J$=1.3 Hz), 8.65 (1H, t, $J$=5.6 Hz), 9.89 (1H, s), 10.29 (1H, br), 11.44 (2H, br); IR $\nu_{max}$ $cm^{-1}$ 1695, 1630.

*Anal* Calcd for $C_{21}H_{23}NO_6$: C 65.44, H 6.01, N 3.63.
Found: C 65.04, H 5.75, N 3.74.

**13**: Yield 73%; yellow crystals (from EtOAc-hexane); mp 224~227°C (dec); $^1$H NMR $\delta$ 1.19 (6H, d, $J$=6.6 Hz), 4.12 (1H, m), 6.29 (2H, d, $J$=8.2 Hz), 7.27 (1H, t, $J$=8.2 Hz), 7.61 (1H, d, $J$=1.4 Hz), 7.91 (1H, d, $J$=1.4 Hz), 8.43 (1H, d, $J$=7.6 Hz), 9.89 (1H, s), 10.25 (1H, br), 11.43 (2H, br); IR $\nu_{max}$ cm$^{-1}$ 1690, 1630.

*Anal* Calcd for $C_{18}H_{17}NO_6$: C 62.97, H 4.99, N 4.08.
Found: C 62.68, H 5.12, N 3.96.

**14**: Yield 57%; yellow crystals (from $CHCl_3$-MeOH); mp 226~229°C (dec); $^1$H NMR $\delta$ 2.30 (3H, br s), 6.31 (2H, d, $J$=8.2 Hz), 7.18 (2H, br d, $J$=8.4 Hz), 7.28 (1H, t, $J$=8.2 Hz), 7.67 (2H, br d, $J$=8.4 Hz), 7.68 (1H, d, $J$=1.4 Hz), 8.04 (1H, d, $J$=1.4 Hz), 9.95 (1H, s), 10.30 (1H, br), 10.39 (1H, br s), 11.45 (2H, br); IR $\nu_{max}$ cm$^{-1}$ 1670, 1620, 1600.

*Anal* Calcd for $C_{22}H_{17}NO_6$: C 67.52, H 4.38, N 3.58.
Found: C 67.18, H 4.20, N 3.52.

**15**: Yield 60%; yellow powder; $^1$H NMR $\delta$ 2.84 (6H, s), 3.28 (2H, br t, $J$=6.0 Hz), 3.66 (2H, br q, $J$=5.8 Hz), 6.33 (2H, d, $J$=8.3 Hz), 7.27 (1H, t, $J$=8.3 Hz), 7.67 (1H, d, $J$=1.2 Hz), 8.02 (1H, d, $J$=1.2 Hz), 9.01 (1H, t, $J$=5.7 Hz), 9.90 (1H, s), 10.16 (1H, br), 10.42 (1H, br), 11.45 (2H, br); IR $\nu_{max}$ cm$^{-1}$ 1700, 1625.

*Anal* Calcd for $C_{19}H_{20}N_2O_6 \cdot HCl \cdot \frac{1}{2}H_2O$: C 54.62, H 5.31, N 6.70, Cl 8.48.
Found: C 54.73, H 5.43, N 6.66, Cl 8.40.

**16**: Yield 72%; yellow crystals (from EtOAc-hexane); mp 200~202°C (dec); $^1$H NMR $\delta$ 1.88 (4H, m), 3.47 (4H, m), 6.28 (2H, d, $J$=8.2 Hz), 7.26 (1H, t, $J$=8.2 Hz), 7.27 (1H, d, $J$=1.4 Hz), 7.60 (1H, d, $J$=1.4 Hz), 9.88 (1H, s), 10.27 (1H, br), 11.47 (2H, br); IR (KBr) cm$^{-1}$ 1680, 1620.

*Anal* Calcd for $C_{19}H_{17}NO_6$: C 64.22, H 4.82, N 3.94.
Found: C 63.78, H 4.69, N 3.85.

**17**: Yield 67%; yellow crystals (from EtOAc-hexane); mp 100~140°C (no well-defined); $^1$H NMR $\delta$ 3.3~3.8 (8H, br), 6.28 (2H, d, $J$=8.2 Hz), 7.16 (1H, d, $J$=1.4 Hz), 7.26 (1H, t, $J$=8.2 Hz), 7.48 (1H, d, $J$=1.4 Hz), 9.88 (1H, s), 10.32 (1H, br), 11.47 (2H, br); IR $\nu_{max}$ cm$^{-1}$ 1620.

*Anal* Calcd for $C_{19}H_{17}NO_7$: C 61.45, H 4.61, N 3.77.
Found: C 61.16, H 4.71, N 3.55.

5-*O*-Methyl Derivative (**19**)

Anhydrous $K_2CO_3$ (42 mg, 0.30 mmol) and dimethyl sulfate (29 $\mu$l, 0.31 mmol) were added to a suspension of **18** (100 mg, 0.28 mmol) in $Me_2CO$ (2.0 ml). After refluxing for 30 minutes, the reaction mixture was filtered, and the filtrate was concentrated to give a crystalline residue. The residue was hydrolyzed with acid by a method similar to that used in the preparation of **9** to afford **19** (65 mg, 74%) as pale yellow crystals (from EtOAc-hexane): MP 210°C (dec); $^1$H NMR $\delta$ 3.82 (3H, s), 6.30 (2H, d, $J$=8.2 Hz), 7.28 (1H, t, $J$=8.2 Hz), 7.84 (1H, d, $J$=1.2 Hz), 8.18 (1H, d, $J$=1.2 Hz), 9.98 (1H, s), 11.42 (2H, br); IR $\nu_{max}$ cm$^{-1}$ 1700, 1635, 1600.

*Anal* Calcd for $C_{16}H_{12}O_7$: C 60.76, H 3.82.
Found: C 60.60, H 3.87.

2'-*O*-Methyl Derivative (**21**)

Anhydrous $K_2CO_3$ (127 mg, 0.92 mmol) and methoxymethyl chloride (70 $\mu$l, 0.92 mmol) were added to a solution of **18** (300 mg, 0.83 mmol) in DMF (3.0 ml). The reaction mixture was stirred at room temperature for 1 hour and diluted with EtOAc (30 ml). The mixture was washed with 1 N hydrochloric acid, water and brine, dried and concentrated. The crystalline residue was chromatographed on silica gel, eluting with hexane-EtOAc (4:1). The pure fraction was concentrated and crystallized from EtOAc-hexane to yield **20** (273 mg, 81%) as yellow crystals: MP 89.5~90°C; $^1$H NMR $\delta$ 3.39 (3H, s), 3.48 (3H, s), 3.49 (3H, s), 5.30 (2H, s), 5.49 (2H, s), 6.30 (1H, s), 6.62 (1H, dd, $J$=8.2 and 1.1 Hz), 6.67 (1H, dd, $J$=8.2 and 1.1 Hz), 7.48 (1H, t, $J$=8.2 Hz), 7.84 (1H, d, $J$=1.4 Hz), 7.89 (1H, d, $J$=1.4 Hz), 11.60 (1H, br); IR $\nu_{max}$ cm$^{-1}$ 1730, 1635, 1615.

*Anal* Calcd for $C_{20}H_{20}O_9$: C 59.41, H 4.99.
Found: C 59.41, H 4.96.

Anhydrous $K_2CO_3$ (103 mg, 0.75 mmol) and dimethyl sulfate (70 $\mu$l, 0.74 mmol) were added to a solution of **20** (100 mg, 0.25 mmol) in $Me_2CO$ (2.0 ml). The reaction mixture was refluxed for 1 hour and filtered. The filtrate was concentrated and the crystalline residue thus obtained was dissolved in THF and

1 N hydrochloric acid (4 : 1, 2.5 ml). The solution was refluxed for 18 hours. The reaction mixture was then worked up in the usual manner to afford **21** (51 mg, 65%) as pale yellow crystals (from EtOAc): MP 217~217.5°C; $^1$H NMR $\delta$ 3.32 (3H, s), 6.45 (1H, dd, $J$=8.3 and 0.8 Hz), 6.59 (1H, dd, $J$=8.3 and 0.8 Hz), 7.45 (1H, t, $J$=8.3 Hz), 7.75 (1H, d, $J$=1.4 Hz), 8.02 (1H, d, $J$=1.4 Hz), 9.92 (1H, s), 10.50 (1H, br), 12.56 (1H, br); IR $\nu_{max}$ cm$^{-1}$ 1700, 1620.

*Anal* Calcd for $C_{16}H_{12}O_7$: C 60.76, H 3.82.
Found: C 60.71, H 3.99.

2′,5-Di-*O*-methyl Derivative (**22**)

Anhydrous $K_2CO_3$ (150 mg, 1.1 mmol) and dimethyl sulfate (132 $\mu$l, 1.4 mmol) were added to a suspension of **18** (100 mg, 0.28 mmol) in $Me_2CO$ (2.0 ml). The reaction mixture was refluxed for 2 hours and filtered. The filtrate was concentrated and the resulting residue was hydrolyzed in the usual manner to give **22** (81 mg, 88%) as pale yellow crystals (from EtOAc): MP 264~266°C (dec); $^1$H NMR $\delta$ 3.30 (3H, s), 3.80 (3H, s), 6.45 (1H, dd, $J$=8.4 and 0.8 Hz), 6.60 (1H, dd, $J$=8.4 and 0.8 Hz), 7.46 (1H, t, $J$=8.4 Hz), 7.86 (1H, d, $J$=1.2 Hz), 8.19 (1H, d, $J$=1.2 Hz), 9.97 (1H, s), 12.50 (1H, s); IR $\nu_{max}$ cm$^{-1}$ 1695, 1630, 1600.

*Anal* Calcd for $C_{17}H_{14}O_7$: C 61.82, H 4.27.
Found: C 61.66, H 4.32.

2′,5,6′-Tri-*O*-methyl Derivative (**23**)

Anhydrous $K_2CO_3$ (400 mg, 2.9 mmol) and dimethyl sulfate (275 $\mu$l, 2.9 mmol) were added to a solution of **5** (100 mg, 0.29 mmol) in $Me_2CO$ (2.0 ml). The reaction mixture was refluxed for 2 hours and filtrated. The filtrate was concentrated to give a crystalline residue. The residue was hydrolyzed in the usual manner to give **23** (56 mg, 54%) as colorless crystals (from EtOAc-hexane): MP 197~200°C (dec); $^1$H NMR $\delta$ 3.62 (6H, s), 3.70 (3H, s), 6.71 (2H, d, $J$=8.4 Hz), 7.41 (1H, t, $J$=8.4 Hz), 7.75 (1H, d, $J$=1.2 Hz), 7.98 (1H, d, $J$=1.2 Hz), 9.95 (1H, s); IR $\nu_{max}$ cm$^{-1}$ 1710, 1600.

*Anal* Calcd for $C_{18}H_{16}O_7$: C 62.79, H 4.68.
Found: C 62.57, H 4.67.

Compound **24**

Compound **4** (400 mg, 1.3 mmol) was treated with dimethylamine hydrochloride, TEA, DCC and HOBT by the same method as that used to prepare **10**. The residue was chromatographed on silica gel, eluting with $CHCl_3$-MeOH (40 : 1). The pure fraction was concentrated, and the residue was crystallized from EtOAc to give yellow crystals of **25** (360 mg, 83%): MP 170~171°C; $^1$H NMR $\delta$ 2.91 (3H, br s), 2.99 (3H, br s), 3.45 (3H, s), 6.10 (1H, s), 6.63 (1H, dd, $J$=8.2 and 1.1 Hz), 6.67 (1H, $J$=8.2 and 1.1 Hz), 6.99 (1H, d, $J$=1.5 Hz), 7.06 (1H, d, $J$=1.5 Hz), 7.50 (1H, t, $J$=8.2 Hz), 10.51 (1H, br); IR $\nu_{max}$ cm$^{-1}$ 1630, 1580.

*Anal* Calcd for $C_{18}H_{17}NO_6$: C 62.97, H 4.99, N 4.08.
Found: C 62.74, H 5.27, N 4.37.

Compound **25** (300 mg, 0.88 mmol) was methylated and hydrolyzed with acid by the same method as that used to prepare **19** to give **24** (257 mg, 86%) as yellow crystals (from EtOAc-hexane): MP 194~196°C (dec); $^1$H NMR $\delta$ 2.98 (3H, br s), 3.04 (3H, br s), 3.78 (3H, s), 6.29 (2H, d, $J$=8.2 Hz), 7.27 (1H, t, $J$=8.2 Hz), 7.44 (1H, d, $J$=1.2 Hz), 7.62 (1H, d, $J$=1.2 Hz), 9.91 (1H, s), 11.46 (2H, br); IR $\nu_{max}$ cm$^{-1}$ 1710, 1625.

*Anal* Calcd for $C_{18}H_{17}NO_6$: C 62.97, H 4.99, N 4.08.
Found: C 62.71, H 5.08, N 3.98.

Chlorination of **1**, Compound **26**

*N*-Chlorosuccinimide (48.6 mg, 0.36 mmol) was added to a solution of **1** (100 mg, 0.33 mmol) in DMF (1.0 ml) and 1 N hydrochloric acid (0.2 ml). The mixture was stirred at room temperature for 1 hour and diluted with EtOAc (30 ml). The solution was washed successively with water and brine, dried and concentrated to give crystals. Recrystallization from EtOAc-hexane afforded orange crystals of **26** (96 mg, 86%): MP 213~216°C (dec); $^1$H NMR $\delta$ 6.26 (1H, d, $J$=8.9 Hz), 7.45 (1H, d, $J$=8.9 Hz), 7.73 (1H, d, $J$=1.3 Hz), 8.03 (1H, d, $J$=1.3 Hz), 9.94 (1H, s), 10.50 (1H, br), 10.71 (1H, br), 13.02 (1H, br), 13.32 (1H,

br); IR $\nu_{max}$ $cm^{-1}$ 1710, 1615.

*Anal* Calcd for $C_{15}H_9O_7Cl \cdot \frac{1}{4}H_2O$: C 52.80, H 2.81, Cl 10.39.
Found: C 52.96, H 2.75, Cl 9.95.

Hydrogenation of **1**, Compound **27**

A solution of **1** (100 mg, 0.33 mmol) in MeOH (10 ml) was hydrogenated over 10% Pd-C (10 mg) under an atmosphere of hydrogen at room temperature for 3 hours. The mixture was filtered and the filtrate was concentrated to give a yellow powder of **27** (57 mg, 57%); $^1H$ NMR $\delta$ 4.37 (2H, s), 5.23 (1H, br), 6.29 (2H, d, $J$=8.2 Hz), 7.27 (1H, t, $J$=8.2 Hz), 7.30 (1H, br s), 7.55 (1H, br s), 9.81 (1H, br), 11.45 (2H, br), 12.80 (1H, br); IR $\nu_{max}$ $cm^{-1}$ 1705, 1630.

*Anal* Calcd for $C_{15}H_{12}O_7 \cdot \frac{1}{4}H_2O$: C 58.35, H 4.08.
Found: C 58.46, H 4.14.

Hydrogenation of **1**, Compound **28**

A solution of **1** (100 mg, 0.33 mmol) was hydrogenated over 10% Pd-C (100 mg) under an atmosphere of hydrogen at room temperature for 23 hours. The mixture was filtered and the filtrate was concentrated. The oily residue was chromatographed on silica gel, eluting with $CHCl_3$-MeOH-AcOH (20:2:1). The pure fraction was concentrated and crystallized from EtOAc-hexane to afford colorless needles of **28** (32 mg, 34%): 215°C (dec); $^1H$ NMR $\delta$ 4.98 (1H, br d, $J$=11.8 Hz), 5.20 (1H, dd, $J$=11.8 and 3.0 Hz), 6.18 (2H, d, $J$=8.1 Hz), 6.66 (1H, br d, $J$=3.0 Hz), 6.81 (1H, t, $J$=8.1 Hz), 7.18 (1H, br s), 7.25 (1H, br s), 9.17 (2H, br), 12.45 (1H, br); IR $\nu_{max}$ $cm^{-1}$ 1700, 1600.

*Anal* Calcd for $C_{15}H_{12}O_6 \cdot \frac{1}{5}H_2O$: C 61.73, H 4.28.
Found: C 61.82, H 4.41.

Oxidation of **1**, Compound **29**

Sulfamic acid (193 mg, 2.0 mmol) and sodium chlorite (33 mg, 0.37 mmol) were added to a solution of **1** (100 mg, 0.33 mmol) in dioxane (2.0 ml) and $H_2O$ (2.0 ml). The mixture was stirred at room temperature for 30 minutes and diluted with water (5 ml). The mixture was extracted with EtOAc, and the organic layer was washed with water, dried and concentrated. The residue was crystallized from EtOAc-hexane to yield pale yellow needles of **29** (82 mg, 70%): MP 230~231°C (dec); $^1H$ NMR $\delta$ 6.26 (1H, d, $J$=8.8 Hz), 7.43 (1H, d, $J$=8.8 Hz), 7.62 (1H, d, $J$=1.4 Hz), 7.95 (1H, d, $J$=1.4 Hz), 10.30 (1H, br), 10.68 (1H, br), 13.14 (2H, br); IR $\nu_{max}$ $cm^{-1}$ 1700, 1620.

*Anal* Calcd for $C_{15}H_9O_8Cl$: C 51.08, H 2.57, Cl 10.05.
Found: C 51.17, H 2.62, Cl 9.07.

Oxime (**30**)

Hydroxylamine hydrochloride (25 mg, 0.36 mmol) was added to a cooled solution of **1** (100 mg, 0.33 mmol) in pyridine (2.0 ml). The mixture was stirred at 0°C for 30 minutes and concentrated. The residue was suspended in EtOAc (20 ml) and washed with 1 N hydrochloric acid and brine. The organic layer was dried and concentrated to give an oily residue. The residue was chromatographed on silica gel, eluting with $CHCl_3$-MeOH-AcOH (20:2:1). The pure fraction was concentrated to afford **30** (71 mg, 68%) as yellow green crystals: MP 90~110°C (dec); $^1H$ NMR $\delta$ 6.29 (2H, d, $J$=8.2 Hz), 7.27 (1H, t, $J$=8.2 Hz), 7.42 (1H, d, $J$=1.1 Hz), 7.70 (1H, d, $J$=1.1 Hz), 10.05 (1H, br), 11.30 (1H, br s); IR $\nu_{max}$ $cm^{-1}$ 1700, 1630.

*Anal* Calcd for $C_{15}H_{11}NO_7 \cdot 1\frac{1}{2}H_2O$: C 52.33, H 4.10, N 4.07.
Found: C 52.23, H 3.51, N 4.39.

*O*-Methyl oxime (**31**) was prepared in a manner similar to that used for the preparation of **30**.

**31**: Yield 71%; pale yellow powder; $^1H$ NMR $\delta$ 3.69 (3H, s), 6.29 (2H, d, $J$=8.2 Hz), 7.27 (1H, t, $J$=8.2 Hz), 7.45 (1H, d, $J$=1.4 Hz), 7.70 (1H, d, $J$=1.4 Hz), 7.99 (1H, s), 10.28 (1H, br), 11.38 (2H, br); IR $\nu_{max}$ $cm^{-1}$ 1700, 1630.

*Anal* Calcd for $C_{16}H_{13}NO_7$: C 58.01, H 3.96, N 4.23.
Found: C 58.33, H 3.99, N 4.27.

Acetylation of **1**, Compound **32**

A solution of **1** (300 mg, 0.99 mmol) in pyridine (3.0 ml) and acetic anhydride (1.5 ml) was stirred at room temperature for 3 hours. The mixture was diluted with EtOAc (50 ml) and washed successively with 3 N hydrochloric acid, water and brine. The organic layer was dried and concentrated to give an oily residue. The residue was chromatographed on silica gel, eluting with $CHCl_3$-MeOH (100 : 1) and the pure fraction was concentrated and crystallized from EtOAc-hexane to afford **32** as pale yellow crystals (253 mg, 66%): MP 167~168°C; $^1$H NMR $\delta$ 1.97 (3H, s), 1.99 (3H, s), 6.68 (1H, br d, $J$=8.2 Hz), 6.79 (1H, br d, $J$=8.2 Hz), 7.45 (1H, t, $J$=8.2 Hz), 8.06 (1H, d, $J$=1.4 Hz), 8.41 (1H, d, $J$=1.4 Hz), 10.00 (1H, s), 10.83 (1H, br s), 13.70 (1H, br); IR $\nu_{max}$ cm$^{-1}$ 1780, 1700, 1630, 1605.

*Anal* Calcd for $C_{19}H_{14}O_9$: C 59.07, H 3.65.
Found: C 59.14, H 3.66.

Acetylation of **1**, Compound **33**

DMAP (60 mg, 0.49 mmol) was added to a solution of **1** (300 mg, 0.99 mmol) in pyridine (3.0 ml) and acetic anhydride (1.5 ml), and the mixture was stirred at room temperature for 24 hours. The mixture was treated in a manner similar to that used for the preparation of **32** to give **33** (203 mg, 48%) as colorless crystals: MP 211~213.5°C; $^1$H NMR $\delta$ 2.02 (3H, s), 2.28 (3H, s), 2.31 (3H, s), 6.95 (1H, dd, $J$=8.3 and 1.1 Hz), 7.02 (1H, dd, $J$=8.3 and 1.1 Hz), 7.41 (1H, s), 7.59 (1H, t, $J$=8.3 Hz), 7.85 (1H, d, $J$=1.3 Hz), 8.23 (1H, d, $J$=1.3 Hz); IR $\nu_{max}$ cm$^{-1}$ 1770, 1750, 1705, 1690, 1610.

*Anal* Calcd for $C_{21}H_{16}O_{10}$: C 58.88, H 3.76.
Found: C 58.97, H 3.72.

Biological Activities

*In vitro* and *in vivo* experiments were performed following the method described in the previous paper[1].

Acknowledgment

We are grateful to Drs. Y. SUGINO, M. NISHIKAWA and H. OKAZAKI for their encouragement throughout this work.

## References

1) ISHII, T.; T. HIDA, T. ISHIMARU, S. IINUMA, K. SUDO, M. MUROI, T. KANAMARU & H. OKAZAKI: TAN-931, a novel nonsteroidal aromatase inhibitor produced by *Penicillium funiculosum* No. 8974. I. Taxonomy, fermentation, isolation, characterization and biological activities. J. Antibiotics 44: 589~599, 1991
2) BALLANTINE, J. A.; D. J. FRANCIS, C. H. HASSALL & J. L. C. WRIGHT: The biosynthesis of phenols. Part XXI. The molecular structure of arugosin, a metabolite of a wild-type strain of *Aspergillus rugulosus*. J. Chem. Soc. (C) 1970: 1175~1182, 1970
3) BAX, A. & G. MORRIS: An improved method for heteronuclear chemical shift correlation by two-dimensional NMR. J. Magn. Reson. 42: 501~505, 1981
4) KESSLER, H.; C. GRIESINGER, J. ZARBOCK & H. R. LOOSLI: Assignment of carbonyl carbons and sequence analysis in peptides by heteronuclear shift correlation via small coupling constants with broadband decoupling in $t_1$ (COLOC). J. Magn. Reson. 57: 331~336, 1984
5) LINDGREN, B. O. & T. NILSSON: Preparation of carboxylic acids from aldehydes (including hydroxylated benzaldehydes) by oxidation with chlorite. Acta Chim. Scand. 27: 888~890, 1973
6) HASHIMOTO, T.; M. TORI & Y. ASAKAWA: Structure of new aromatic compounds isolated from ascocarps of a species of Xylariaceae. Abstracts of Papers 56th Annual Meeting of the Chemical Society of Japan, No. 1IM40, Kyoto, Apr. 1~4, 1989
7) BRODIE, A. M. H.; W. M. GARRETT, J. R. HENDRICKSON & C.-H. TSAI-MORRIS: Effects of aromatase inhibitor 4-hydroxyandrostenedione and other compounds in the 7,12-dimethylbenz(*a*)anthracene-induced breast carcinoma model. Cancer Res. 42 (Suppl.): S3360~S3364, 1982
8) SANTEN, R. J.; S. SANTNER, B. DAVIS, J. VELDHUIS, E. SAMOJLIK & E. RUBY: Aminoglutethimide inhibits extraglandular estrogen production in postmenopausal women with breast carcinoma. J. Clin. Endocrinol. Metab. 47: 1257~1265, 1978
9) SANTEN, R. J.; E. SAMOJLIK & S. A. WELLS: Resistance of the ovary to blockade of aromatization with

aminoglutethimide. J. Clin. Endocrinol. Metab. 51: 473~477, 1980

10) KELLIS, J. T., Jr. & L. E. VICKERY: Inhibition of human estrogen synthetase (aromatase) by flavones. Science 225: 1032~1034, 1984

11) KELLIS, J. T., Jr.; S. NESNOW & L. E. VICKERY: Inhibition of aromatase cytochrome P-450 (estrogen synthetase) by derivatives of α-naphthoflavone. Biochem. Pharmacol. 35: 2887~2891, 1986

12) MASON, J. I.; B. A. MURRY, M. OLCOTT & J. J. SHEETS: Imidazole antimycotics: Inhibitors of steroid aromatase. Biochem. Pharmacol. 34: 1087~1092, 1985

13) SCHIEWECK, K.; A. S. BHATNAGAR & A. MATTER: CGS 16949A, a new nonsteroidal aromatase inhibitor: Effects on hormone-dependent and -independent tumors *in vivo*. Cancer Res. 48: 834~838, 1988