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

I. TAXONOMY, FERMENTATION, ISOLATION, CHARACTERIZATION AND BIOLOGICAL ACTIVITIES

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A novel nonsteroidal aromatase inhibitor, TAN-931, was isolated from the culture filtrate of a soil isolate fungus, No. 8974. The strain was identified as *Penicillium funiculosum* No. 8974. TAN-931 inhibited human placental and rat ovarian aromatase activity, and the IC_{50} value was 17.2 and 162 μM, respectively. The inhibition of human placental aromatase was uncompetitive with respect to androstenedione conversion with a *Ki* value of 40 μM. When TAN-931 was subcutaneously administered at doses of 25, 50 and 100 mg/kg (once/day, $\times$4) to 20-day-old female Sprague-Dawley rats treated with gonadotropin, the plasma estradiol-17β level and the weight of ovaries and uterus were markedly reduced in a dose-dependent manner. The *in vivo* inhibitory activity of TAN-931 was more potent than that of 4-hydroxyandrostenedione.

Consecutive administration of TAN-931 (100 mg/kg, sc, twice/day, $\times$7) to 9-week-old male Sprague-Dawley rats did not induce any adrenal hypertrophy even though administration of aminoglutethimide caused 2-fold enlargement of the adrenal under the same conditions.

Specific binding of TAN-931 to the estrogen receptor from a human breast cancer cell line, MCF-7, was not detected.

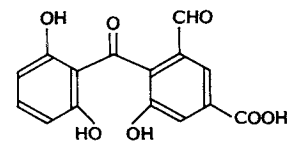
Estrogen acts not only as a stimulatory hormone, increasing the mitotic activity in the target tissues, but also as a primary risk factor in the development of the estrogen-dependent tumors[1]. For example, estrogen synthesized either in the ovary or extraglandular sites stimulates the proliferation of estrogen-dependent metastatic breast cancer in pre- or post-menopausal women. Thus, in the treatment of breast cancer patients, it seems to be an effective therapeutic approach to deprive the tumor of estrogen using aromatase inhibitors such as aminoglutethimide (AG)[2,3] and 4-hydroxyandrostenedione (4-OHA)[4~8] or to antagonize the function of estrogen at the receptor site using antiestrogens such as tamoxifen[9].

Aromatase is an NADPH-dependent monooxygenase, and a member of the cytochrome P-450 family[10]. It catalyzes the conversion of androgen to estrogen and thus is a key enzyme in the glandular and extraglandular biosynthesis of estrogen[11~13]. Aromatase is found predominantly in the pre-menopausal ovary and also in adipose tissue, liver, brain, placenta and breast cancer tissue[14~20]. Inhibitors with a high specificity for aromatase should therefore lower the level of circulating estrogen without affecting the

† Concerning patents on these subjects, refer to Jpn. Kokai 131588 ('90), May 21, 1990 [Eur. Pat. 342 665, Nov. 23, 1989].

biosynthesis of other steroids. In view of this hypothesis, we screened various microbial metabolites in search of aromatase-specific inhibitors and found such a compound in a culture of a soil isolate fungus. The active substance isolated from the culture broth, TAN-931, was shown to be a strong inhibitor of aromatase activity from human placenta, and its structure was elucidated as 4-(2,6-dihydroxybenzoyl)-3-formyl-5-hydroxybenzoic acid which was proved to be a novel compound (Fig. 1). This report describes the taxonomy of the producing organism and the fermentation, isolation, characterization and biological activities of TAN-931.

Fig. 1. Structure of TAN-931.



Materials and Methods

Taxonomic Studies

Culture characterization was carried out following the classifications described by MALLOCH[21] and RAPER *et al.*[22].

Fermentation

A loopful of spores and mycelia of strain No. 8974 grown on an agar slant was inoculated into a 2-liter flask containing 500 ml of a sterile seed medium consisting of glucose 2% (w/v), maltose 3%, soybean flour 1.5%, corn steep liquor 1%, Polypepton 0.5%, yeast extract 0.3% and NaCl 0.3%, pH 6.0. The flask was shaken on a reciprocal shaker at 28°C for 40 hours. The entire seed culture was transferred to a 50-liter fermenter containing 30 liters of the same medium supplemented with 0.05% (w/v) Actocol (Takeda Chemical Ind., Japan). Cultivation was carried out at 28°C for 48 hours under aeration of 30 liters/minute and agitation of 280 rpm. One liter of the seed culture thus obtained was transferred to a 200-liter fermenter containing 100 liters of a production medium consisting of glucose 1% (w/v), dextrin 4%, soybean flour 0.5%, Polypepton 0.5%, malt extract 0.5%, yeast extract 0.2%, K_2HPO_4 1% and $CaCO_3$, pH 7.0 before sterilization. Cultivation was carried out at 24°C for 90 hours under aeration of 100 liters/minute and agitation of 150 rpm.

Assay for TAN-931 Production

The amount of TAN-931 in the fermentation broth was determined by HPLC using a Lichrospher 100 PR-18(e) (5 μm, Merck) with a mobile phase of MeOH - 0.01 M phosphate buffer (20 : 80, pH 7.0).

Isolation of TAN-931

The culture filtrate (20 liters) was extracted with EtOAc (2 × 10 liters) at pH 3.2. The EtOAc layer was extracted with 2% aq $NaHCO_3$ (2 × 7 liters). The aqueous layer was adjusted to pH 3.1 and re-extracted with EtOAc (2 × 8 liters). The EtOAc layer was washed with water and concentrated *in vacuo* to give an oily residue. Silica gel (50 g) mixed with the residue was placed on the top of the silica gel (450 g) column, eluting with $CHCl_3$ - MeOH - acetic acid (40 : 1 : 0.5 → 7 : 1 : 0.5). The fractions containing TAN-931 were combined and concentrated *in vacuo* to give a crystalline residue, which was recrystallized from $CHCl_3$ - MeOH to give orange crystals (1.36 g).

Analytical Measurement

MP's were uncorrected. UV spectra were taken on a Hitachi 320 spectrophotometer. IR spectra were obtained with a Hitachi 285 grating IR spectrophotometer. EI-MS spectra were measured on a Jeol JMS-DX303 instrument.

Preparation of Human Placental and Rat Ovarian Aromatase

Microsome fraction as an aromatase source was prepared from human placenta or rat ovaries accord-

ing to the method of THOMPSON and SIITERI[10].

Measurement of the Inhibition of Aromatase Activity

Aromatase activity was determined according to the method of THOMPSON and SIITERI[10] by measuring the conversion of [1β,2β-^{3}H]androstenedione to ^3H_2O and unlabeled estrone. Briefly, the reaction mixture (225 μl) consisting of [^{3}H]androstenedione (140,000 dpm, 4 μM), NADPH (550 μM), potassium phosphate buffer (pH 7.4, 20 mM) and human placental microsomal protein (20 μg) was incubated at 37°C for 1 hour and then extracted with 0.5 ml of chloroform. Residual steroid in the aqueous fraction was further adsorbed with 0.25 ml of 5% activated charcoal (Norit A), and the radioactivity of an aliquot of the supernatant (10,000 × g, 5 minutes) was determined using a liquid scintillation counter. Percent inhibition of aromatase was calculated by dividing the radioactivity in the presence of inhibitors by that in the absence of inhibitors. For the determination of *Ki* or the type of the inhibition, NADPH was replaced with an NADPH-generating system consisting of NADP 1.3 mM, glucose-6-phosphate 9.8 mM and glucose-6-phosphate dehydrogenase 0.25 units.

In Vivo Evaluation of TAN-931

TAN-931 was suspended in 0.2% gum arabic in saline and subcutaneously injected into 20-day-old female Sprague-Dawley (SD) rats at doses of 25, 50 and 100 mg/kg once a day for 4 days. On the third day, rats were given a subcutaneous injection of 10 units of pregnant mare serum gonadotropin (PMSG). On the fifth day, rats were anesthetized with ether, blood was collected from the abdominal aorta using heparinized syringes, and the uterus and both ovaries were excised and weighed. The amount of estradiol-17β in the plasma was measured by a radioimmuno assay as follows. Blood samples were centrifuged at 3,000 × g for 5 minutes and the plasma (0.4 ml) was extracted with 2 ml of ethyl ether. Aliquots (0.5 ml) of the ether extract were taken and dried under a nitrogen stream. A 0.1-ml portion of 50 mM Tris-HCl (pH 8.0) containing 0.1 M NaCl, 0.1% NaN_3 and 0.1% gelatin (binding buffer) was added to the residue, and this was mixed using a vortex mixer. Anti-estradiol-17β serum (0.5 ml) was added, and, after incubation at room temperature for 30 minutes, one hundred μl of [2,4,6,7-^{3}H]estradiol-17β (3.3 nCi) was added to the reaction mixture. After incubating at 37°C for 1 hour, the mixture was chilled in ice bath for 15 minutes, and the binding buffer (0.2 ml) containing 0.05% Dextran T-70 and 0.5% activated charcoal (Norit A) was added. After 10-minute incubation in the ice bath, the dextran-coated charcoal was removed by centrifugation at 10,000 × g for 2 minutes, and the radioactivity in the supernatant (0.25 ml) was measured using a liquid scintillation counter. Assays were performed in duplicate.

Effect of TAN-931 on the Weight of Various Organs in Rats

TAN-931 was administered to 9 week-old male SD rats to determine whether or not it induced adrenal hypertrophy. TAN-931 was suspended in polypropyleneglycol - saline (1 : 1) and subcutaneously injected (100 mg/kg, twice/day, total 7 times), and, 1 hour after the final dose, rats were decapitated. The pituitary, adrenal and liver were excised and weighed. AG and 4-OHA were used as positive controls.

Affinity of TAN-931 for the Estrogen Receptor

Human breast cancer cell line, MCF-7, was the kind gift of Dr. M. E. LIPPMAN (NCI). The binding affinity of TAN-931 for the cytosolic estrogen receptor was examined according to the method of BRUEGGEMEIER and KATLIC[23] with slight modification. MCF-7 cells were grown in 150-cm^2 plastic flasks at 37°C in EAGLE's minimum essential medium (MEM) supplemented with dextran-charcoal treated 10% fetal calf serum (FCS)[24]. Cells from subconfluent cultures were harvested by treatment with 0.1% trypsin and 0.02% EDTA, washed once with phosphate-buffered saline and suspended in 5 ml of 10 mM Tris - HCl (pH 7.4) containing 1.5 mM EDTA and 1 mM dithiothreitol. Cells (1.1×10^8) were disrupted using a Polytron homogenizer (Kinematica, GmbH) and the homogenate was centrifuged at 100,000 × g for 1 hour. The resulting supernatant was used as the source of cytosolic estrogen receptors. The cytosolic protein (177 μg) was incubated at 4°C for 16 hours with 2 nM of [^{3}H]estradiol-17β in a total volume of 250 μl in the absence or presence of various concentrations of unlabeled estradiol-17β, tamoxifen or TAN-931. The amount of receptor-bound [^{3}H]estradiol-17β was also determined according to the method of BRUEGGEMEIER and KATLIC[23]. The specific binding was calculated by subtracting the total binding from the binding in the

presence of 1 μM unlabeled estradiol-17β.

Other Assay

Protein was determined according to the method of BRADFORD[25].

Chemicals

[1β,2β-^{3}H]-4-Androstene-3,17-dione (48 Ci/mmol) and [2,4,6,7-^{3}H]estradiol-17β (100 Ci/mmol) were purchased from New England Nuclear (Boston, MA), and 4-androstene-3,17-dione, estradiol-17β, tamoxifen (*trans*-isomer), AG, Norit A activated charcoal were obtained from Sigma Chemical Company (St. Louis, MO). NADPH, NADP, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were purchased from Oriental Yeast Co., Ltd. (Osaka, Japan), PMSG from Teikoku Zouki Co., Ltd. (Tokyo, Japan), estradiol-17β-6-BSA antiserum (rabbit) from Seikagaku-kogyo Co., Ltd. (Tokyo, Japan), 4-OHA from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), Dextran T-70 from Pharmacia (Sweden), and FCS and MEM from M. A. Bioproducts (Walkersville, MD). All other chemicals used were from commercial sources and of reagent grade.

Animals

SD rats were obtained from CLEA Japan, Inc., and immature female SD rats were used for all experiments at 20 days of age.

Statistical Analysis

Student's t-test was used to determine the statistical significance.

Results

Taxonomy of the Producing Organism

Strain No. 8974 was isolated from a soil sample collected in Sayou-cho, Hyogo Prefecture, Japan. Growth of strain No. 8974 on malt extract agar medium was as follows: string-like mycelia form a colony having a slightly raised surface with an irregular periphery, the aerial hyphae develop well and formation of conidia is slightly inferior, the center of the colony is dark green and the periphery is yellow, and the reverse is pale tan.

According to the taxonomic properties summarized in Table 1 and the features of penicilli shown in Fig. 2, strain No. 8974 was proved to belong to the Biverticillata-Symmetrica Section of the genus *Penicillium*. The colonies on CZAPEK's agar, malt extract agar, potato-glucose agar and oatmeal agar showed following features: Perithecia were not produced, colonies were not strongly coremiform,

Table 1. Taxonomic properties of strain No. 8974.

Colony surface	Funiculose
Conidiophore	Irregularly branched from aerial hyphae
Penicilli	Biverticillate and symmetrical
Metulae	1.5~2.0×9~10 μm, cylindrical, 6~8 metulae in the vertical
Phialides	1.5~2.0×9~10 μm, cylindrical with acuminate tips, 6~8 phialides in the vertical on metulae
Conidia	1.5×2.2 μm, elliptical

Fig. 2. Scanning electron micrograph of spore-bearing penicilli of strain No. 8974 developing on yeast extract-malt extract agar.

Magnification, ×4,000.

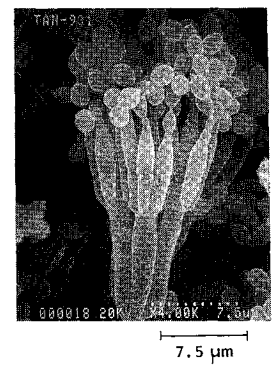


Fig. 3. Time course of TAN-931 production.

● TAN-931, ▲ pH.

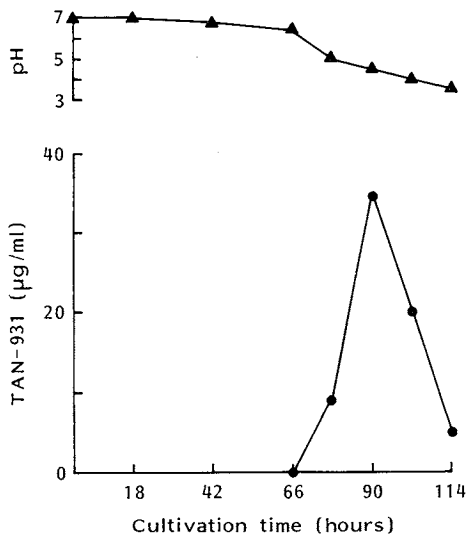


Table 2. Physico-chemical properties of TAN-931.

Appearance	Orange crystals
MP	241~244°C (dec)
EI-MS (*m/z*)	302 (M^+)
Molecular formula	$C_{15}H_{10}O_7$
Anal Calcd:	C 59.61, H 3.33, (O 37.06)
Found:	C 59.59, H 3.41
UV λ_{max}^{MeOH} nm (ε)	223 (31,100), 275 (13,300), 336 (6,950)
$\lambda_{max}^{0.02\,N\,NaOH\text{-}MeOH}$ nm (ε)	235 (sh, 24,800), 275 (sh, 11,600), 385 (5,620)
IR (KBr) cm^{-1}	3600~2500, 1720, 1630, 1595

the colony surface was funiculose. Therefore, the strain was identified as *Penicillium funiculosum* No. 8974 and has been deposited in the Institute for Fermentation, Osaka under the accession No. IFO 32067. The strain was also deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, with the accession No. FERM BP-1873.

Fermentation

A typical example of the time course of fermentation is shown in Fig. 3. The amount of TAN-931 in the culture filtrate reached about 35 μg/ml at 90 hours, but its rapid decrease was observed with further cultivation of strain No. 8974.

Isolation and Characterization

Since TAN-931 is a fat-soluble, acidic compound, it was isolated by a usual method for such products. The acidified culture filtrate was extracted with EtOAc, and then the organic layer was extracted with aq $NaHCO_3$. The aqueous layer was acidified and re-extracted with EtOAc. The organic layer was washed with water and concentrated. The residue was chromatographed on a silica gel column, and active fractions were concentrated to give crude crystals. Recrystallization from $CHCl_3$-MeOH gave TAN-931 as orange crystals.

TAN-931 is soluble in MeOH, EtOH, Me_2CO and DMSO, slightly soluble in $CHCl_3$ and ethyl ether, but insoluble in water and *n*-hexane. It reacts with 2,4-dinitrophenylhydrazine, $FeCl_3$ and Tollens reagents but not with ninhydrin or Greig-Leaback reagents.

The physico-chemical properties of TAN-931 are summarized in Table 2. The molecular formula was determined to be $C_{15}H_{10}O_7$ by elemental analysis and the EI-MS spectrum. The structure was elucidated as 4-(2,6-dihydroxybenzoyl)-3-formyl-5-hydroxybenzoic acid, which will be reported in the following paper[26].

Biological Activities

Inhibition of human placental and rat ovarian aromatase activity by TAN-931 was measured. The inhibitory activity (IC_{50} value) of TAN-931 against human and rat enzymes was 17.2 and 162 μM, respectively (Fig. 4). In comparison with AG and 4-OHA used as the positive controls, the inhibitory activity of TAN-931 was more potent than that of AG in the case of human aromatase (Fig. 4A), while 4-OHA was

Fig. 4. Inhibition of aromatase activity.

Human placental (A) aromatase (222 fmol/minute) or rat ovarian (B) aromatase preparations (146 fmol/minute) were incubated at 37°C for 1 hour with varying concentrations of AG (○), 4-OHA (●) or TAN-931 (▲) and the amount of ^3H_2O released was measured as described in Materials and Methods. Assays were performed in duplicate.

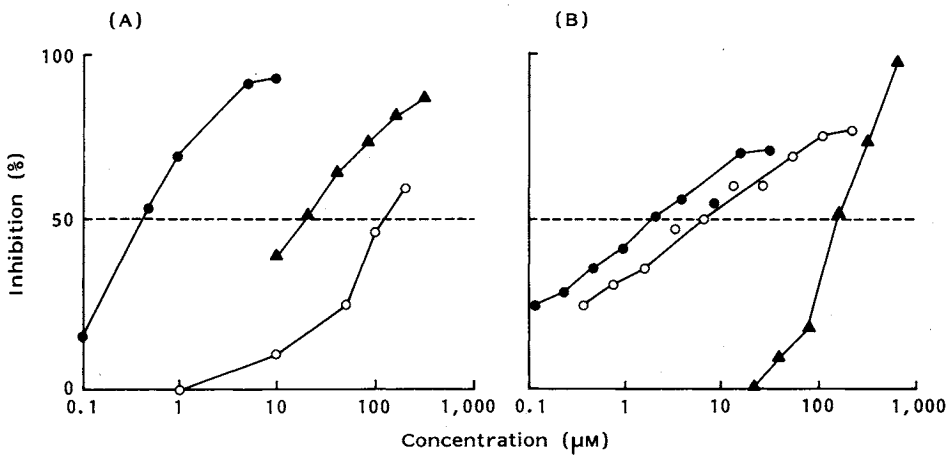
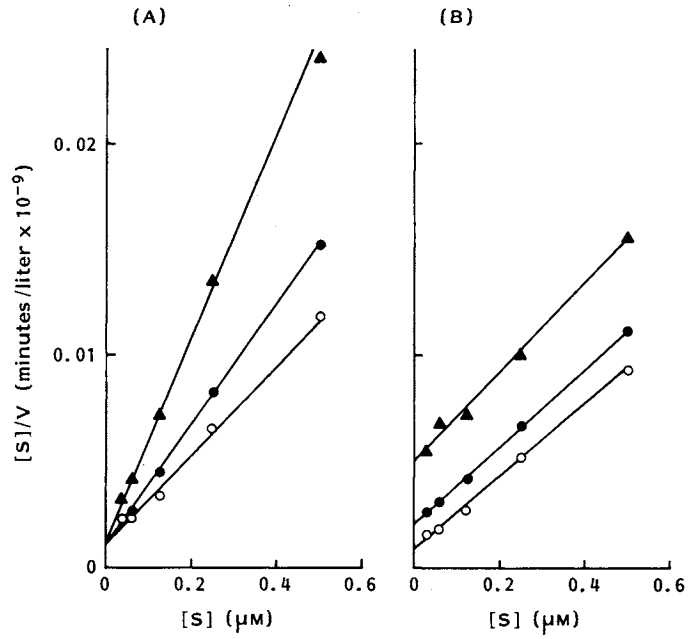


Fig. 5. Hanes-Woolf plot of the inhibition by 4-OHA or TAN-931 as a function of substrate concentration.

(A) TAN-931; ○, none; ●, 13.2 μM; ▲, 53 μM. *Ki* 40 μM.
(B) 4-OHA; ○, none; ●, 6.3 nM; ▲, 25 nM. *Ki* 7.8 nM.

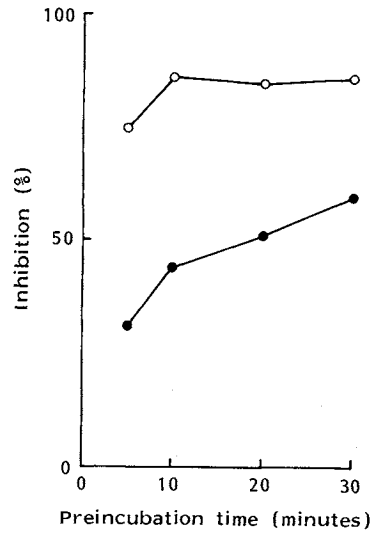


Human placental aromatase preparation (222 fmol/minute) was incubated at 37°C for 1 hour with the various concentrations of [^{3}H]androstenedione (28,000 dpm) indicated in the figure. Assays were performed in duplicate.

the most potent *in vitro* inhibitor of aromatase from both sources. The kinetics of the inhibition by TAN-931 was examined using the microsomal fraction of human placenta, as described in Materials and Methods. Hanes-Woolf plots[27] revealed an uncompetitive type of inhibition with respect to androstenedione conversion with a *Ki* value of 40 μM (Fig. 5A). The mode of inhibition was different from that of 4-OHA, which caused a mixed type inhibition with a *Ki* value of 7.8 nM (Fig. 5B). Preincubation of aromatase with TAN-931 or 4-OHA, prior to the addition of [^{3}H]androstenedione, resulted in a time-dependent increase in the aromatase inhibitory activity for both compounds (Fig. 6).

Fig. 6. Time-dependent increase in the inhibition caused by 4-OHA and TAN-931.

Human placental aromatase preparation (222 fmol/minute) was preincubated at 37°C for the indicated time with 50 nM 4-OHA (○) or 17 μM TAN-931 (●) in the absence of substrate, and then 1 μM [^{3}H]androstenedione (140,000 dpm) was added, and the mixture was further incubated at 37°C for 1 hour.



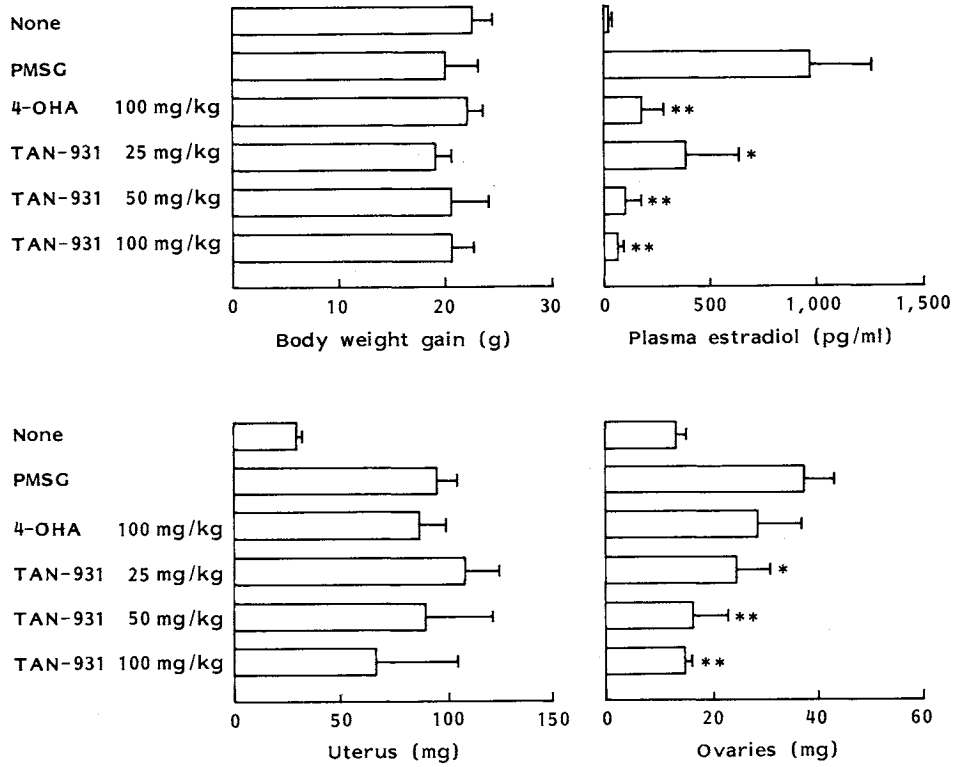
Inhibition of estrogen biosynthesis by TAN-931 was evaluated *in vivo*. Gonadotrophic stimulation of the immature female SD rat caused uterine and ovarian hypertrophy, and elevation of the plasma estrogen level. The plasma estrogen level reached a maximum 2 days after a single sc injection of PMSG, while the weight of the uterus and ovary continued to increase (unpublished data). The *in vivo* effectiveness of TAN-931 was evaluated two days after PMSG stimulation. When TAN-931 was subcutaneously injected at doses of 25, 50 or 100 mg/kg, the PMSG-induced increase in the weight of ovaries and plasma estradiol-17β level was reduced in a dose-dependent manner, being abolished at doses higher than 50 mg/kg (Fig. 7). The weight of the uterus was only slightly, and not significantly, reduced, in spite of the reduction of the plasma estradiol-17β level to the unstimulated level. TAN-931 was shown to be more potent than 4-OHA *in vivo* (Fig. 7). We also measured the plasma estradiol-17β level and the aromatase activity in the ovary of TAN-931-treated immature rats and detected a reduction in total and specific aromatase activity, correlating with the reduction in the plasma estradiol-17β level (Table 3). The reduction of specific aromatase activity indicates that TAN-931 not only suppressed ovarian growth but also inhibited ovarian aromatase *in vivo*.

The effect of TAN-931 on various organs was examined after 4-day administration as described in Materials and Methods (Table 4). AG treatment induced adrenal hypertrophy, as previously reported[28~30], and liver enlargement but did not exert any effect on body weight gain. 4-OHA reduced the weight of adrenals, probably due to its hormonal activity, but did not exert any effect on other organs. TAN-931 induced a significant increase in body weight but did not cause adrenal hypertrophy. These data indicate that TAN-931 specifically inhibits aromatase activity in a manner distinct from that seen with AG.

The effect of TAN-931 on specific binding of radiolabeled estradiol-17β to estrogen receptors from MCF-7 cells was examined. The apparent *Kd* value for estradiol-17β was found to be 0.37 nM, and the number of specific binding sites was 6,700 sites per cell when analyzed by the SCATCHARD[31] procedure (unpublished data). As shown in Fig. 8, unlabeled estradiol-17β and tamoxifen could displace radiolabeled

Fig. 7. Effect of TAN-931 on the weight of uterus and ovaries and on the plasma estradiol-17β level.

* $P<0.01$, ** $P<0.001$ against PMSG-group.



4-OHA or TAN-931 was subcutaneously injected once a day for four days into 20-day-old female Sprague-Dawley rats (5 rats per group). Rats were treated with PMSG (10 U/rat) on day 3 and, on the fifth day, rats were sacrificed.

estradiol-17β from the high affinity binding sites (ED_{50}, 5 and 4,000 pM, respectively), whereas TAN-931 did not displace it at concentrations up to 0.1 mM, indicating that TAN-931 does not have affinity for estrogen receptors.

Discussion

A novel fungal metabolite, TAN-931, was found to inhibit aromatase from human placenta and rat ovary. Investigation into the kinetics of the inhibition revealed that TAN-931, unlike 4-OHA, uncompetitively inhibited the aromatase activity from human placenta (Fig. 5). TAN-931 inhibited human placental aromatase activity (IC_{50}, 17.2 μM) preferably to rat ovarian aromatase activity (IC_{50}, 162 μM). As shown in Fig. 4(A), the inhibition by

Table 3. Effect of TAN-931 on the plasma estradiol-17β level and ovarian aromatase activity.

Compound	Plasma estradiol-17β (pg/ml)	Ovarian aromatase	
		Total (unit)	Specific (unit/mg)
None	29.8±10.0	1.1	12.1
PMSG	213.7±53.7	8.3	62.0
TAN-931 (×3)[a]	98.1±61.1*	3.8	29.4
TAN-931 (×2)[b]	127.6±34.0*	2.9	26.1

Mean±SD, n=6.

Student's t-test against PMSG-group, * $P<0.01$.

PMSG (10 U) was sc injected on day 2 and rats were sacrificed on day 4.

TAN-931 was sc injected at a dose of 100 mg/kg/day.

[a] Three times (day 1, 2 and 3).

[b] Two times (day 2 and 3).

One unit of aromatase activity was expressed as fmol ^3H_2O formed/minute/ovary and specific activity was expressed as unit/mg ovarian microsomal protein.

Table 4. Effect of AG, 4-OHA and TAN-931 on the weight of the pituitary, adrenal and liver.

Compound	Body weight (g)		Organ weight/body weight at day 3		
	Day 1	Day 3	Pituitary (mg/kg)	Adrenal (mg/kg)	Liver (g/kg)
None	331±8	333±10	31.8±3.0	96.5±12.1	43.0±3.4
AG	332±9	328±17	31.4±1.7	187.9±24.4***	47.8±4.9*
4-OHA	331±8	338± 8	33.3±2.9	82.7± 7.9**	43.0±2.7
TAN-931	331±9	361± 7***	29.0±2.4	89.1± 8.2	44.4±2.4

Mean±SD, n=8.
Student's t-test against None-group, * $P<0.05$, ** $P<0.02$, *** $P<0.001$.

TAN-931 was stronger than that by AG. Furthermore, preincubation of human placental aromatase with 4-OHA or TAN-931 reduced aromatase activity in a time-dependent manner in both cases (Fig. 6). This may suggest that TAN-931 irreversibly inactivates aromatase.

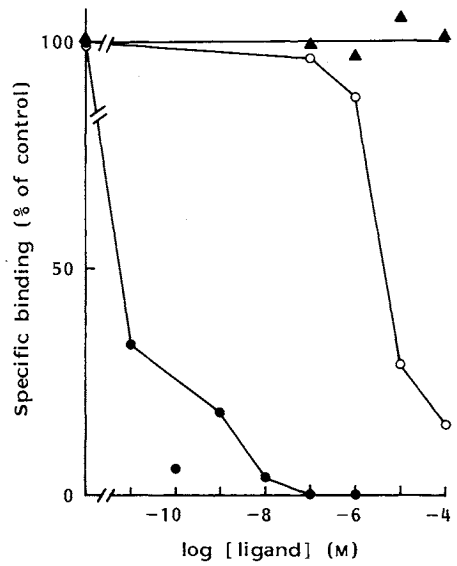
Subcutaneous administration of TAN-931 to female rats markedly inhibited estrogen biosynthesis *in vivo*. The *in vivo* inhibitory activity of TAN-931 was very strong and more potent than that of 4-OHA (Fig. 7), although the IC_{50} value of 4-OHA was 40-fold lower than that of TAN-931.

AG strongly inhibits "desmolase" (cholesterol side-chain cleavage enzyme) and induces adrenal hypertrophy by nonselectively blocking steroidogenesis[29~31]. TAN-931, however, did not exert any effect on the adrenal (Table 4). This shows that TAN-931, unlike AG, appears to be relatively specific for aromatase.

TAN-931 has no affinity for estrogen receptors (Fig. 8), and does not inhibit the proliferation of MCF-7 cells at concentrations up to 0.32 mM (data not shown). It is improbable that TAN-931 binds directly to the estrogen receptors and attenuates estrogenic functions *in vivo* in addition to inhibiting estrogen biosynthesis.

Fig. 8. Competition of tamoxifen or TAN-931 with estradiol-17β for MCF-7 estrogen receptors.

The binding of tamoxifen or TAN-931 to MCF-7 cytosolic estrogen receptors was determined by examining the competition of tamoxifen (○), TAN-931 (▲) or unlabeled estradiol-17β (●) with 2 nM [^{3}H]estradiol-17β at the indicated concentrations.



This is the first report of an aromatase inhibitor of microbial origin. Since toxicity of TAN-931 was not observed at doses lower than 800 mg/kg (ICR male mice, 4-week-old, sc), TAN-931 may prove to be a potent therapeutic agent for treating estrogen-dependent diseases such as breast cancer.

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