R1128 SUBSTANCES, NOVEL NON-STEROIDAL ESTROGEN-RECEPTOR ANTAGONISTS PRODUCED BY A *Streptomyces*

II. PHYSICO-CHEMICAL PROPERTIES AND STRUCTURE DETERMINATION

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R1128 A, B, C and D, new non-steroidal estrogen-receptor antagonists, were isolated from the cultured broth of *Streptomyces* sp. No. 1128. Their structures were elucidated to be 1,3,6-trihydroxy-8-alkylanthraquinones on the basis of their physico-chemical properties and spectroscopic data.

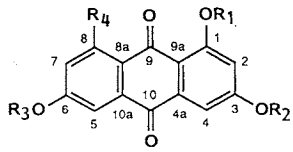
R1128 A, B, C and D are new non-steroidal estrogen-receptor antagonists. In the preceding paper[1], we described the taxonomy of the producing strain as well as the fermentation, isolation and biological properties of these substances. In this paper, we report the physico-chemical properties and structure determination of R1128 substances (Fig. 1).

Results

Physico-chemical Properties of R1128 Substances

The physico-chemical properties of R1128 A, B, C and D are summarized in Table 1. While they were isolated all as orange powders, R1128 A was slightly brownish and R1128 D was slightly yellowish. The molecular formulas of R1128 A, B, C and D were established to be $C_{17}H_{14}O_5$, $C_{18}H_{16}O_5$, $C_{19}H_{18}O_5$ and $C_{19}H_{18}O_5$, respectively, by HRFAB-MS and ^{13}C NMR. The IR spectrum, ^1H NMR spectrum and ^{13}C NMR spectrum of R1128 B, the most abundant component, are shown in Figs. 2, 3 and 4, respectively. R1128 substances are soluble in methanol, ethanol, acetone, ethyl acetate, chloroform, diethyl ether and acetonitrile, while insoluble in *n*-hexane and water. They showed positive color reactions to iodine vapor, ceric sulfate, sulfuric acid and ferric chloride, but negative to ninhydrin, EHRLICH, MOLISCH and

Fig. 1. Structures of R1128 substances.



R1128 A (**1**)	$R_1=R_2=R_3=H$	$R_4=CH_2CH_2CH_3$
R1128 B (**2**)	$R_1=R_2=R_3=H$	$R_4=CH_2CH_2CH_2CH_3$
R1128 C (**3**)	$R_1=R_2=R_3=H$	$R_4=CH_2CH_2CH(CH_3)_2$
R1128 D (**4**)	$R_1=R_2=R_3=H$	$R_4=CH_2CH_2CH_2CH_2CH_3$
Triacetyl R1128 B (**2a**)	$R_1=R_2=R_3=COCH_3$	$R_4=CH_2CH_2CH_2CH_3$

Table 1. Physico-chemical properties of R1128 substances.

	1	2	3	4
Appearance	Brownish-orange powder	Orange powder	Orange powder	Yellowish-orange powder
MP	238~243°C (dec)	250~255°C (dec)	240~245°C	237~242°C
Molecular formula	$C_{17}H_{14}O_5$	$C_{18}H_{16}O_5$	$C_{19}H_{18}O_5$	$C_{19}H_{18}O_5$
HRFAB-MS $(M+H)^+$				
Calcd:	299.0919	313.1076	327.1232	327.1232
Found:	299.0931	313.1092	327.1216	327.1216
UV λ_{max}^{MeOH} nm (log ε)	204 (4.2), 220 (4.3), 270 (sh, 4.2), 286 (4.4), 309 (sh, 4.0), 347 (3.5), 434 (3.7)	203 (4.3), 218 (4.4), 269 (sh, 4.3), 285 (4.5), 306 (sh, 4.2), 347 (3.6), 433 (3.7)	202 (4.3), 217 (4.5), 265 (sh, 4.4), 284 (4.6), 307 (sh, 4.2), 343 (3.7), 432 (3.8)	203 (4.3), 219 (4.5), 270 (sh, 4.4), 286 (4.6), 309 (sh, 4.2), 342 (3.7), 436 (3.8)
$\lambda_{max}^{0.01\text{ N HCl-MeOH}}$ nm (log ε)	222 (4.3), 271 (sh, 4.2), 287 (4.4), 311 (sh, 3.9), 348 (3.5), 433 (3.7)	219 (4.4), 270 (sh, 4.3), 285 (4.5), 310 (sh, 4.1), 347 (3.6), 433 (3.8)	218 (4.5), 266 (sh, 4.4), 284 (4.6), 308 (sh, 4.1), 344 (3.7), 430 (3.9)	219 (4.5), 269 (sh, 4.4), 285 (4.6), 310 (sh, 4.1), 346 (3.7), 432 (3.8)
$\lambda_{max}^{0.01\text{ N NH}_4\text{OH-MeOH}}$ nm (log ε)	231 (4.2), 261 (4.0), 269 (sh, 4.0), 311 (4.5), 386 (3.6), 505 (3.7)	230 (4.3), 259 (4.1), 266 (sh, 4.1), 310 (4.6), 385 (3.7), 503 (3.8)	229 (4.4), 258 (4.2), 266 (sh, 4.2), 309 (4.7), 384 (3.8), 502 (3.8)	229 (4.4), 258 (4.2), 265 (sh, 4.2), 309 (4.7), 383 (3.8), 501 (3.9)
IR ν_{max}^{KBr} cm^{-1}	3400, 2960, 2930, 2860, 1665, 1630, 1600, 1570, 1500, 1460, 1400, 1320, 1260, 1230, 1180, 1160, 1140, 1110, 1080, 1020, 1000, 950	3400, 2960, 2930, 2860, 1665, 1630, 1600, 1570, 1500, 1480, 1460, 1400, 1330, 1310, 1280, 1260, 1240, 1200, 1160, 1130, 1110, 1080, 1020, 980, 900	3400, 2960, 2930, 2860, 1660, 1630, 1600, 1570, 1500, 1460, 1400, 1360, 1320, 1260, 1240, 1180, 1160, 1140, 1080, 1020, 990, 900	3400, 2960, 2940, 2860, 1660, 1630, 1600, 1570, 1500, 1460, 1400, 1320, 1260, 1240, 1180, 1160, 1140, 1080, 1020, 990, 960, 900
TLC Rf value				
System I[a]	0.52	0.53	0.55	0.55
System II[b]	0.47	0.39	0.32	0.31

[a] Plate; Silica gel 60 F_{254} (E. Merck, Art. 5715), solvent; *n*-hexane-acetone (1 : 1).

[b] Plate; RP-18 $F_{254}S$ (E. Merck, Art. 13724), solvent; 70% aq CH_3CN.

Fig. 2. IR spectrum of R1128 B in KBr.

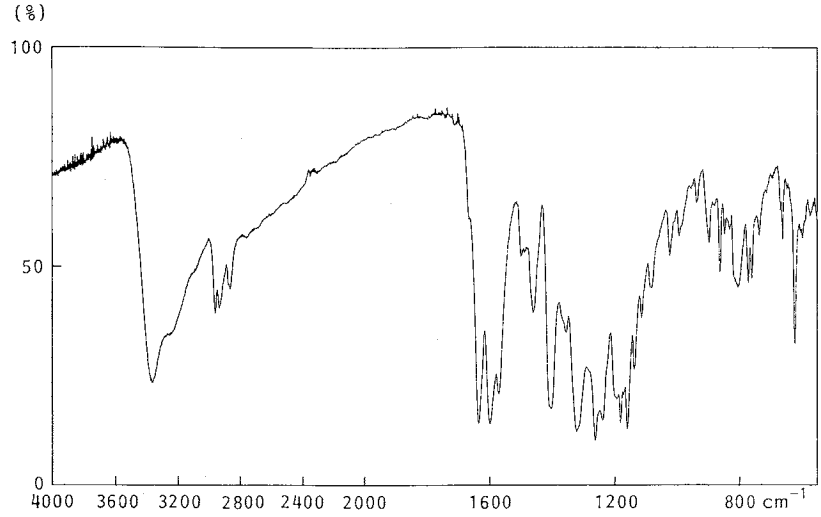


Fig. 3. ^{1}H NMR spectrum of R1128 B in CD_3OD (400 MHz).

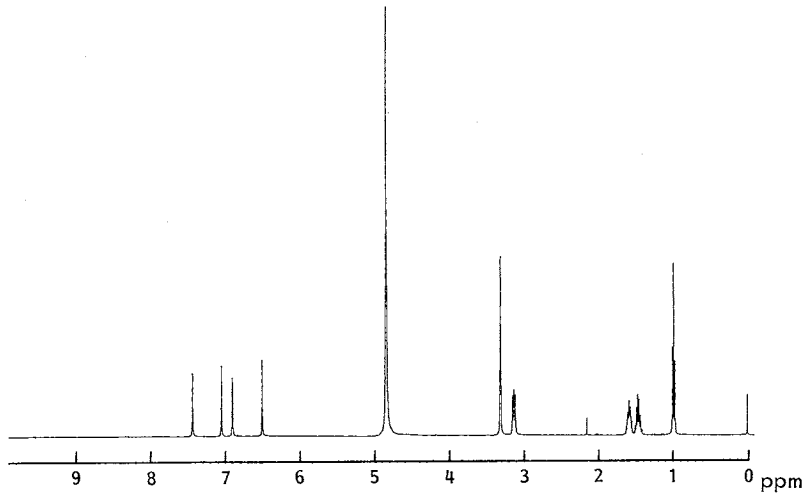
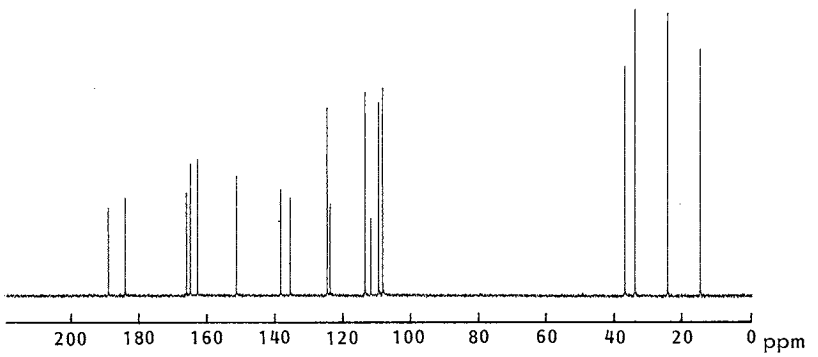


Fig. 4. ^{13}C NMR spectrum of R1128 B in CD_3OD (100 MHz).



DRAGENDORFF reagents. The Rf values of R1128 A, B, C and D on silica gel TLC developed with *n*-hexane - acetone (1 : 1) were 0.52, 0.53, 0.55 and 0.55, respectively, and those on RP-18 TLC developed with 70% aqueous acetonitrile were 0.47, 0.39, 0.32 and 0.31, respectively.

Structure Determination of R1128 Substances

Structure of R1128 B (**2** in Fig. 1)

Initially, the structural efforts were focused on R1128 B, as it was the most abundant component. HRFAB-MS measurement yielded the molecular formula of **2** to be $C_{18}H_{16}O_5$, which was consistent with ^{13}C NMR data (Table 2). In ^{13}C NMR spectrum, the two carbon signals at δ_C 188.9 (s) and 184.0 (s) were quite characteristic of quinone carbonyls. Inspection of ^1H NMR spectrum (400 MHz, CD_3OD) of **2** revealed the presence of two sets of *meta*-coupling spin systems on the aromatic rings [δ_H 6.46 (1H, d, J=2 Hz), 7.01 (1H, d, J=2 Hz); 6.85 (1H, d, J=2 Hz), 7.40 (1H, d, J=2 Hz)]. The NMR informations, eleven degrees of unsaturation and orange color appearance of **2** reminded us of an anthraquinone chromophore in the molecule of **2**.

The examination of ^1H NMR and ^{13}C NMR spectrum of **2** also indicated the presence of a *n*-butyl group [δ_H 3.09 (2H, m), 1.55 (2H, m), 1.45 (2H, m) and 0.96 (3H, t, J=6 Hz); δ_C 36.7 (t), 33.7 (t), 24.0 (t) and 14.4 (q)].

Acetylation of **2** gave triacetyl derivative (**2a**) [FAB-MS m/z 439 $(M+H)^+$] in high yield. The chemical shifts of three acetyl protons (δ_H 2.47, 2.36 and 2.35) in combination with strong absorption at 1770 cm^{-1} in the IR spectrum indicated the presence of three phenol-acetyl groups in **2a**.

These findings suggested that **2** has an anthraquinone chromophore substituted by two sets of *meta*-coupled protons, one *n*-butyl group and three hydroxyl groups. The remaining problem,

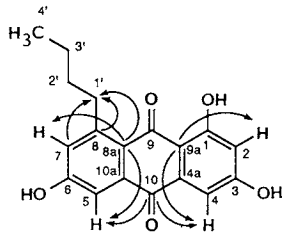
Table 2. ^1H and ^{13}C NMR data for R1128 substances in CD_3OD.

Position	δ_H (400 MHz)				δ_C (100 MHz)			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1					166.2 (s)	165.9 (s)	166.2 (s)	166.2 (s)
2	6.43 (1H, d, 2)	6.46 (1H, d, 2)	6.41 (1H, d, 2)	6.42 (1H, d, 2)	109.3 (d)	109.3 (d)	109.3 (d)	109.3 (d)
3					165.2 (s)	164.8 (s)	165.2 (s)	165.2 (s)
4	6.96 (1H, d, 2)	7.01 (1H, d, 2)	6.94 (1H, d, 2)	6.95 (1H, d, 2)	108.1 (d)	108.1 (d)	108.2 (d)	108.1 (d)
4a					135.8 (s)	135.4 (s)	135.7 (s)	135.7 (s)
5	7.35 (1H, d, 2)	7.40 (1H, d, 2)	7.31 (1H, d, 2)	7.33 (1H, d, 2)	113.4 (d)	113.3 (d)	113.4 (d)	113.4 (d)
6					163.0 (s)	162.6 (s)	163.0 (s)	163.0 (s)
7	6.83 (1H, d, 2)	6.85 (1H, d, 2)	6.78 (1H, d, 2)	6.80 (1H, d, 2)	125.0 (d)	124.5 (d)	124.9 (d)	124.9 (d)
8					151.2 (s)	151.2 (s)	151.8 (s)	151.5 (s)
8a					123.8 (s)	123.6 (s)	123.7 (s)	123.8 (s)
9					189.4 (s)	188.9 (s)	189.2 (s)	189.3 (s)
9a					111.8 (s)	111.7 (s)	111.8 (s)	111.8 (s)
10					184.2 (s)	184.0 (s)	184.1 (s)	184.2 (s)
10a					138.6 (s)	138.2 (s)	138.6 (s)	138.6 (s)
1′	3.03 (2H, m)	3.09 (2H, m)	2.99 (2H, m)	3.01 (2H, m)	39.1 (t)	36.7 (t)	41.2 (t)	37.0 (t)
2′	1.58 (2H, m)	1.55 (2H, m)	1.39 (2H, m)	1.55 (2H, m)	25.0 (t)	33.7 (t)	35.1 (t)	33.3 (t)
3′	0.99 (3H, t, 6)	1.45 (2H, m)	1.65 (1H, m)	1.38 (4H, m)	14.6 (q)	24.0 (t)	29.7 (d)	31.6 (t)
4′		0.96 (3H, t, 6)	0.96 (6H, d, 6)			14.4 (q)	22.9 (q)	23.5 (t)
5′				0.92 (3H, t, 6)			22.9 (q)	14.4 (q)

Chemical shifts are given in ppm. Proton intensities, multiplicity and coupling constants (J in Hz) are given in parentheses.

which was the substitution pattern of **2**, was clarified by analyses of long-range ^{13}C-^{1}H coupling relationships derived from COLOC (Fig. 5). The quinone carbonyl carbon at δ_C 184.0 (C-10) was long-range coupled with aromatic protons both at δ_H 7.01 (H-4, d, J=2 Hz) and 7.40 (H-5, d, J=2 Hz), each of which was previously shown to couple with another aromatic protons at δ_H 6.46 (H-2, d, J=2 Hz) or 6.85 (H-7, d, J=2 Hz), respectively. On the other hand, the aromatic carbons at δ_C 124.5 (C-7), 151.2 (C-8) and 123.6 (C-8a) were long-range coupled with the methylene protons at δ_H 3.09 (H-1'). This result indicated that the *n*-butyl group was located at C-8 position. Thus, the substitution pattern of **2** was inevitably deduced as shown in Fig. 5.

Fig. 5. The key ^{13}C-^{1}H long-range coupling relationships from a COLOC spectrum of R1128 B (**2**).



The quinone carbonyl at δ_C 188.9 (C-9) was suggested to hydrogen-bonded to an phenol proton binding to an aromatic carbon [δ_C 165.9 (C-1, s)]. The strong absorptions at 1665 cm^{-1} and 1630 cm^{-1} in the IR spectrum of **2** were ascribable to non-hydrogen-bonded and hydrogen-bonded quinone carbonyl, respectively.

Further support for the structure of **2** was obtained from the close similarity between UV data of **2** [λ_{max}^{MeOH} nm (log ε) 285 (4.5), 433 (3.7)] and those reported for 1,3,6-trihydroxy-8-methylanthraquinone [λ_{max}^{MeOH} nm (log ε) 284 (4.2), 432 (3.4)][2].

Consequently, the structure of **2** was determined to be 1,3,6-trihydroxy-8-*n*-butylanthraquinone† as shown in Fig. 1.

Structure of R1128 A (**1** in Fig. 1)

The structures of other minor congeners, R1128 A (**1**), R1128 C (**3**) and R1128 D (**4**), were elucidated by comparison of their physico-chemical properties and spectroscopic data with those of **2**. The only difference between **2** and **1**, **3**, **4** was found in the portion of alkyl side chains.

The molecular formula of **1** was established to be $C_{17}H_{14}O_5$ on the basis of HRFAB-MS. The ^{1}H NMR and ^{13}C NMR data of **1** showed the signals due to *n*-propyl group [δ_H 3.03 (2H, m), 1.58 (2H, m) and 0.99 (3H, t, J=6 Hz); δ_C 39.1 (t), 25.0 (t) and 14.6 (q)].

Thus, the structure of **1** was elucidated to be 1,3,6-trihydroxy-8-*n*-propylanthraquinone.

Structure of R1128 C (**3** in Fig. 1)

The molecular formula of **3** was established to be $C_{19}H_{18}O_5$ by HRFAB-MS. The ^{1}H NMR and ^{13}C NMR of **3** suggested the presence of isopentyl group [δ_H 2.99 (2H, m), 1.39 (2H, m), 1.65 (1H, m) and 0.96 (6H, d, J=6 Hz); δ_C 41.2 (t), 35.1 (t), 29.7 (d) and 22.9 (q)×2]. This was also confirmed by the ^{1}H-^{1}H COSY spectral analysis of **3** (data not shown).

Accordingly, the structure of **3** was assigned as 1,3,6-trihydroxy-8-(3-methylbutyl)-anthraquinone.

Structure of R1128 D (**4** in Fig. 1)

The molecular formula of **4** was established as $C_{19}H_{18}O_5$ based on HRFAB-MS and ^{13}C NMR data.

† According to IUPAC nomenclature rule, **2** should be named as 1-*n*-butyl-3,6,8-trihydroxyanthraquinone. In order to avoid unnecessary confusions in numbering system between **2** and **1**, **3**, **4**, however, we adopted this numbering system.

The ^1H NMR and ^{13}C NMR data revealed a *n*-pentyl residue [δ_H 3.01 (2H, m), 1.55 (2H, m), 1.38 (4H, m) and 0.92 (3H, t, $J=6$ Hz); δ_C 37.0 (t), 33.3 (t), 31.6 (t), 23.5 (t) and 14.4 (q)].

Similarly, the structure of **4** was determined to be 1,3,6-trihydroxy-8-*n*-pentylanthraquinone.

Discussion

R1128 A, B, C and D are novel non-steroidal estrogen-receptor antagonists isolated from the cultured broth of *Streptomyces* sp. No. 1128[1]. Their structures are 1,3,6-trihydroxy-8-alkylanthraquinone derivatives as shown in Fig. 1.

A large number of compounds containing 1,3,6-trihydroxyanthraquinone moiety have been isolated from both plants and microorganisms, such as monoamine oxidase inhibitors[3], biosynthetic intermediates of sterigmatocystin and aflatoxin B_1[4], pigments[5] and other metabolites[2,6,7]. 1,3,6-Trihydroxy-8-alkylanthraquinone derivatives like R1128 substances, however, are uncommon except 1,3,6-trihydroxy-8-methylanthraquinone[2].

It might be interesting to examine whether 1,3,6-trihydroxy-8-methylanthraquinone and other compounds containing 1,3,6-trihydroxyanthraquinone moiety show inhibitory activity against estrogen-receptor binding.

Experimental

General

IR spectra were recorded on a Perkin-Elmer 16PC FTIR spectrophotometer. ^1H and ^{13}C NMR spectra were measured on a Bruker AM400WB spectrometer. The chemical shifts are given in ppm (δ) relative to TMS as the internal standard. UV spectra were measured on a Hitachi 220A spectrophotometer. HRFAB- and FAB-MS were measured on a VG ZAB-SE mass spectrometer. MP's were measured with a Yanagimoto Co. microscope hot-stage apparatus and are uncorrected. TLC was performed on pre-coated Silica gel 60 F_{254} plates (Art. 5715, E. Merck, Darmstadt, F.R.G.) and RP-18 $F_{254}S$ plates (Art. 13724, E. Merck).

Acetylation of 2

To a solution of **2** (30 mg) in pyridine (0.8 ml) was added acetic anhydride (0.92 ml) and the reaction mixture was stirred overnight at room temperature. After removal of solvent under N_2 stream, the residue was purified by preparative TLC developed with $CHCl_3$ to give **2a** (39 mg): FAB-MS *m/z* 439 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ_H 1.00 (3H, t, $J=7$ Hz), 1.36~1.70 (4H, m), 2.35 (3H, s), 2.36 (3H, s), 2.47 (3H, s), 3.14 (2H, t, $J=7$ Hz), 7.24 (1H, d, $J=2.5$ Hz), 7.31 (1H, d, $J=2.5$ Hz), 7.88 (1H, d, $J=2.5$ Hz), 7.92 (1H, d, $J=2.5$ Hz); IR ($CHCl_3$) cm^{-1} 1770, 1675, 1600.

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