Synthesis and Antitumor Activity of Tropolone Derivatives. 3

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As part of a study on the antitumor activities of tropolone derivatives prepared from hinokitiol (**1**), which naturally occurs in the plants of *Chamaecyparis* species, effects of aromatic substituents of α,α-bis(7-hydroxy-5-isopropyltropon-2-yl)toluenes (**4**) on the activity were examined. Several of the compounds showed high potency in the P388 leukemia assay. 4-Hydroxy analogue **4d** showed the most potent activity (T/C = 195%) at a 5 mg/kg dose. The introduction of large-size substituents, of which the steric influence prevents coplanarity of the substituted aromatic function, resulted in a remarkable decrease in the potency. X-ray structural analysis of highly potent 4-methoxy analogue **4b** was undertaken.

Our previous reports[1-4] described the syntheses of mono- and bistropolone derivatives (**3** and **4**) from hinokitiol,[5] which naturally occurs in the plants of *Chamaecyparis* species, and their antitumor activities. Although both tropolone derivatives (**3**, **4**) exhibit respective potent inhibitory activities against the growth of KB cells, the activities of bistropolones **4** are about 200 times those of monotropolones **3** in the survival test by use of P388 mice. The previous investigation[3,4] on the molecular modification of **3** and **4** provided evidence that the molecular feature essential for the antitumor activity is the tropolone moiety. In addition, it was found that the presence of two tropolone moieties in a molecule remarkably enhances the antitumor activity in the in vivo system.[3]

These findings led us to focus on the bistropolones **4**, of which the potent activities were considered to be based on this peculiar structural feature. In the present study, we have explored an improved method for the preparation of bistropolones **4** and synthesized a number of bistropolones **4**, having various substituents on the benzene ring, to investigate effects of their substituents on the activity in the in vivo system. We also have replaced the benzene ring in **4** with a heteroaromatic ring. On the other hand, the X-ray structural analysis of highly potent **4b**[3] has been undertaken to elucidate its three-dimensional structure.

Chemistry. We previously reported[2] that hinokitiol (**1**) on treatment with 1.2 molar equiv of benzaldehyde diethyl acetal (**2**) at 150–160 °C gives mono- and bistropolones (**3** and **4**) but that similar reaction of **1** with 0.5 molar equiv of **2** at 180 °C affords **4** in 30–35% yields in pure form with a slight amount of **3** (method A).

Recently, it has been found that the latter reaction often afforded a small amount of a new compound, 1,2-bis(7-hydroxy-5-isopropyltropon-2-yl)-1,2-diphenylethanes (**5**), as a byproduct, and that bistropolones **4** could not be obtained in some cases in the reaction of acetals (**2**) having special substituents. Moreover, purification of **4** produced by the method A was often troublesome, because the byproducts (**3** and/or **5**) and polymeric products were accompanied. After various screening of the reaction conditions, it was found that treatment of **1** with 0.5 molar equiv of **2** in refluxing xylene in the presence of a catalytic amount of potassium *tert*-butoxide and hexamethylphosphoramide (HMPA) gave **4** with selectivity in about 40% yield (method B).

In order to elucidate effects of substituents on the benzene ring of **4** on the potency of the antitumor activity, **4c–r** listed in Table I were synthesized by the methods A and B. Exceptionally, 4-hydroxy analogue **4d** was obtained by the catalytic reduction of 4-benzyloxy analogue **4c** (method C), because heating of a mixture of **1** and 4-hydroxybenzaldehyde acetal (**2d**) gave not **4d** but a complex mixture, likely formed by the self-condensation of the acetal. Consequently, amino analogue **4f**, with a potent electron-releasing group similar to a hydroxy group, was prepared by the hydrolysis of acetamide analogue **4e** (method D). While yields generally were 20% or better, the reaction of **1** with **2h**, having an electron-withdrawing group such as a methoxycarbonyl, resulted in the production of **4h** in poor yield (9%). Similarly, the reaction of **1** with 4-nitro-, 4-cyano-, or 4-carboxybenzaldehyde acetal did not give bistropolone analogue **4**. Carboxy analogue **4i** was obtained by the hydrolysis of the methyl ester **4h** (method E).

The synthesis of heteroaromatic analogues by the reaction of **1** with heteroaromatic aldehyde acetals was attempted. According to the method A, heating of a mixture of **1** and thiophene-2-carboxaldehyde acetal (**2p**) gave **4p** and **5p** in 13 and 3% yields, respectively. Compound **4p** was also successfully obtained in a 25% yield by the reaction in the presence of the catalyst (method B). Similarly, furan analogue **4q** was obtained only when the catalyst was used. However, reaction of **1** with pyridine-4-carboxaldehyde acetal was unsuccessful.

These present findings showed that this reaction of **1** with acetal to give **4** was not applicable to acetals having a potent electron-releasing or potent electron-withdrawing group.

X-ray Crystal Structure. Crystal data of compound **4b** are as follows: crystal system, monoclinic; space group, $P2_1/a$; centrosymmetric; a = 21.8758 (0.0035), b = 17.2765 (0.0021), c = 6.3319 (0.0011) Å; β = 95.311 (0.0114)°; V = 2382.79 (0.63) Å^3; number of reflections, 3260; final R value, 0.096. The X-ray crystal structure of **4b** is shown in Figure 1.

The bond lengths 1.26 Å for C_2–O_1 and 1.36 Å for C_3–O_2 showed that C_2–O_1 forms a carbonyl group while O_2 is part of the hydroxyl group of the tropolone moiety. Similarly, it was found that $C_{2'}$–$O_{1'}$ forms a carbonyl group and $O_{2'}$ is part of the hydroxyl group by their bond lengths.

The benzene ring (A ring) forms an angle of 80.1° with one tropolone ring (B ring) and an angle of 89.9° with the other tropolone ring (C ring). The two tropolone rings (B and C rings) form an angle of 91.8°.

Biological Results and Discussion

Compounds were evaluated for inhibition of growth of

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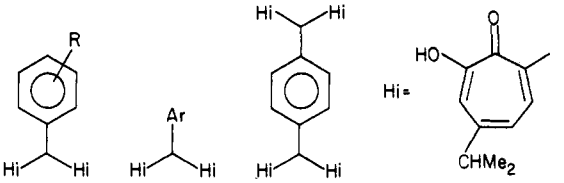
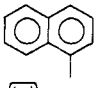
(1) Yamato, M.; Ishikawa, T.; Ishikawa, S.; Hashigaki, K. *Chem. Pharm. Bull.* **1983**, *31*, 2952.
(2) Yamato, M.; Hashigaki, K.; Kokubu, N.; Nakato, Y. *J. Chem. Soc., Perkin Trans. 1* **1984**, 1301.
(3) Yamato, M.; Hashigaki, K.; Kokubu, N.; Tsuruo, T.; Tashiro, T. *J. Med. Chem.* **1984**, *27*, 1749.
(4) Yamato, M.; Hashigaki, K.; Ishikawa, S.; Kobubu, N.; Inoue, Y.; Tsuruo, T.; Tashiro, T. *J. Med. Chem.* **1985**, *28*, 1026.
(5) Nozoe, T. *Bull. Chem. Soc. Jpn.* **1936**, *11*, 295.

0022-2623/86/1829-1202$01.50/0

Table I. Antitumor Activities of Bistropolone Derivatives **4**



4a-n **4o-q** **4r**

compd	R or Ar	method[a]	mp, °C	yield, %	formula	inhibn of KB cell growth, μg/mL	antitumor act. P388 in mice, ip[b] doses, mg/kg	T/C, %
4a	H	A (6)	199–200	34	*c*	<0.3	10	0
							5	188
							2.5	150
							1.3	132
4b	4-OMe	B (12)	204–205	42	*c*	<0.3	20	103
							10	170
							5	173
							2.5	134
4c	4-OCH_2Ph	A (3)	203–204	42	$C_{34}H_{34}O_5$	4.65	100	152
							50	148
							20	101
4d	4-OH	C	219–220	91	$C_{27}H_{28}O_5$	<0.3	10	0
							5	195
							1.3	139
4e	4-NHCOMe	A (7)	152–154	21	$C_{29}H_{31}NO_5$	<0.3	20	0
							10	128
							5	171
4f	4-NH_2	D	244–245	66	$C_{27}H_{29}NO_4$	NT[d]	20	147
							10	147
							5	138
4g	4-NMe_3I	*e*	143–145	95	$C_{30}H_{36}INO_4$	2.5	20	91
							10	95
							5	102
4h	4-COOMe	A (30)	204–206	9	$C_{29}H_{30}O_6$	0.5	20	0
							10	128
							5	145
4i	4-COOH	E	256–257	82	$C_{28}H_{28}O_6$	0.5	20	0
							10	139
							2.5	154
							1.3	124
4j	4-OCH_2COOEt	A (3)	163–164	43	$C_{31}H_{34}O_7$	NT	20	0
							10	0
							5	125
4k	4-OCH_2COONa	*e*	>300	73	$C_{29}H_{29}NaO_7$	2.6	10	0
							5	109
							2.5	115
4l	4-Ph	A (4)	197–198	33	$C_{33}H_{32}O_4$	2.5	20	127
							10	138
							5	111
4m	3,4-OCH_2O	B (12)	214–215	41	$C_{28}H_{28}O_6$	0.5	20	186
							10	193
							5	182
4n	3,4,5-$(OMe)_3$	A (3)	155–156	17	$C_{30}H_{34}O_7$	<0.3	20	162
							10	151
							5	142
4o		B (12)	235–236	33	$C_{31}H_{30}O_4$	2.6	20	178
							10	178
							5	180
4p		B (12)	183–184	25	$C_{25}H_{20}O_4S$	0.6	20	0
							10	57
							5	178
4q		B (12)	114–116	24	$C_{25}H_{26}O_5$	NT	10	0
							5	177
							2.5	141
							1.3	131
4r		A (12)	276–277	19	$C_{48}H_{50}O_8$	100	20	102
							10	105
							5	107

[a] The Arabic numerals in parentheses show reaction time. [b] The dose listed was given once a day for 1 and 5 days. [c] See ref 2. [d] NT = not tested. [e] See the Experimental Section for details.

KB cells[6] and antitumor activity against leukemia P388 in mice.

In our previous study,[3] activities of analogues 4 with no substituent, methyl, methoxy, dimethylamino, or chloro

Scheme I

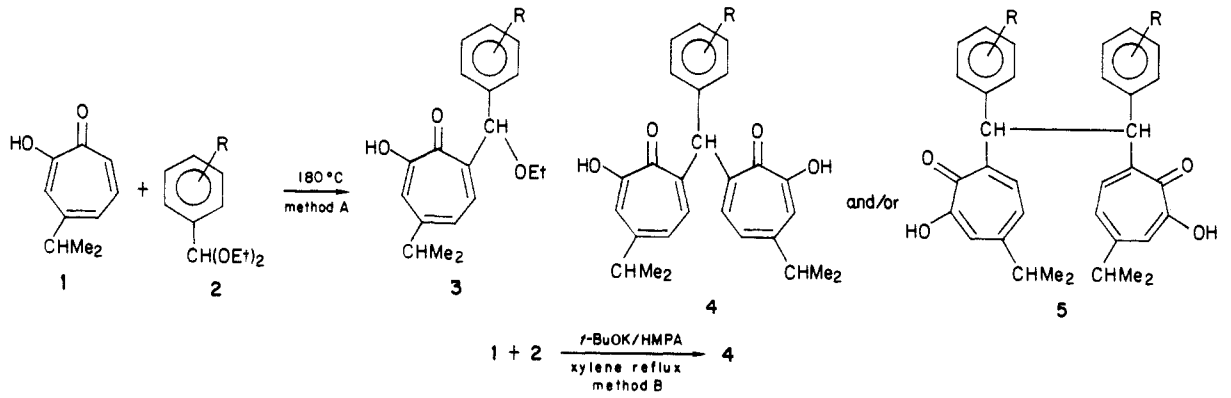
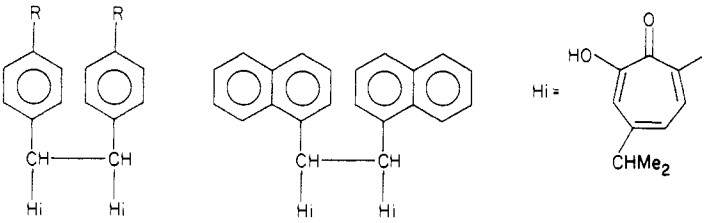


Table II. Antitumor Activities of Diphenylethenes 5



compd	R	mp, °C	yield,[a] %	formula	inhibn of KB cell growth, μg/mL	antitumor act. P388 in mice, ip[b] doses, mg/kg	T/C, %
5b	OMe	257–259	2	$C_{36}H_{38}O_6$	5.2	400	139
						200	140
						100	138
5c	OCH_2Ph	247–248	3	$C_{48}H_{46}O_6$	<100	NT[c]	
5o		279–281	8	$C_{42}H_{38}O_4$	<100	400	95
						200	96
						100	98

[a] The compound was obtained as a byproduct, when bistropolone 4 was prepared by method A. [b] See Table I. [c] NT = not tested.

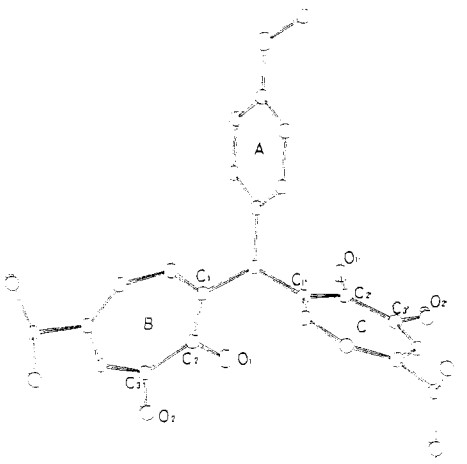


Figure 1. X-ray crystal structure of **4b**.

group, on the benzene ring were examined. With the exception that the 4-chloro analogue is somewhat weaker, the electronic properties and the positions of these substituents do not give a remarkable effect on the potency of the activity in the in vivo system.

In the present study, 4-hydroxy analogue **4d** showed the most potent activity at a 5 mg/kg dose. 4-Methoxycarbonyl (**4h**) and 4-carboxy (**4i**) analogues were also found to be somewhat weaker than unsubstituted **4a**. Interestingly, analogues having an ionic substituent such as trimethylammonium iodide (**4g**) or sodium carboxylate (**4k**) were inactive.

The introduction of a large-size substituent such as benzyloxy (**4c**) or phenyl (**4l**) on the para position of benzene ring resulted in a remarkable decrease in the potency, suggesting that the steric influence of these substituents prevents coplanarity of the substituted aromatic function. In contrast to them, 3,4-methylenedioxy (**4m**) and naphthalene (**4o**) analogues possessed a larger aromatic function but showed comparable activity to highly potent compound **4a** in a range of 5–20 mg/kg doses. Thiophene (**4p**) and furan (**4g**) analogues were essentially equivalent in activity to benzene analogue **4a**.

It was very interesting that **4r** having four tropolone moieties in a molecule was inactive. In addition, in a series of diphenylethanes **5**, methoxy analogue **5b** showed remarkably weak activity and benzyloxy (**5c**) and naphthalene (**5o**) analogues were inactive, in spite of the presence of two tropolone moieties in their molecules. These results indicated that analogues having two hinokitiol moieties are not always active and that the stereospecific structural feature of bistropolones **4** is the most favorable for the antitumor activity. X-ray structural analysis of **4b** reveals that the benzene and two tropolone rings are at nearly right angles each other. This steric arrangement (at least in the crystal) may play an important role in the antitumor action.

In summary, steric characteristics of substituents on the benzene ring of bistropolones **4** alter the potency of the

(6) Tsuruo, T.; Iida, H.; Tsukagoshi, S.; Sakurai, Y. *Cancer Res.* 1979, *39*, 1063.

activity. The present results for the molecular modification and stereochemistry of **4b** suggested that the unique structural feature of bistropolones **4** would be implicated in the mechanism of the antitumor action.

Experimental Section

Melting points are uncorrected. NMR spectra were run on a Hitachi R-24 spectrometer at 60 MHz, with Me_4Si as an internal standard. MS were recorded on a Shimadzu LKB-9000 spectrometer. The elemental analysis are within 0.4% of the theoretical values.

Synthesis of α,α-Bis(7-hydroxy-5-isopropyltropon-2-yl)toluenes (4). Method A. A mixture of 4-(benzyloxy)benzaldehyde diethyl acetal (**2c**; 3.5 g, 12 mmol) and hinokitiol (**1**; 1.4 g, 24 mmol) was heated at 180 °C for 3 h under an argon atmosphere and chromatographed on silica gel (Wako gel C-300). Elution with hexane–AcOEt (4:1) gave successively 4-(benzyloxy)-α,α-bis(7-hydroxy-5-isopropyltropon-2-yl)toluene (**4c**; 2.7 g, 42%) and 1,2-bis(7-hydroxy-5-isopropyltropon-2-yl)-1,2-bis[4-(benzyloxy)phenyl]ethane (**5c**; 0.26 g, 3% based on **2c**). **4c**: mp 203–204 °C (from CH_2Cl_2); NMR ($CDCl_3$) δ 1.26 (12 H, d, J = 7 Hz, 2 CH*Me*$_2$), 2.50–3.20 (2 H, m, 2 C*H*Me$_2$), 5.09 (2 H, s, OCH_2), 6.68 (1 H, s, CH), 6.70–7.70 (15 H, m, tropolone H and aromatic H), 9.10–9.20 (2 H, br s, 2 OH); MS, m/e 522 (M^+). Anal. ($C_{34}H_{34}O_5$) C, H. **5c**: mp 247–248 °C (from CH_2Cl_2); NMR (CF_3COOD) δ 1.41 (12 H, d, J = 7 Hz, 2 CH*Me*$_2$, 2.80–3.40 (2 H, m, 2 C*H*Me$_2$, 5.18 (4 H, s, 2 CH_2Ph, 6.39 (2 H, s, 2 CH), 6.80–9.20 (24 H, m, tropolone H and aromatic H); MS, m/e 718 (M^+). Anal. ($C_{48}H_{46}O_6$) C, H.

Method B. A solution of **1** (5 g, 31 mmol), piperonal diethyl acetal (**2n**; 3.4 g, 15 mmol), t-BuOK (0.25 g, 2.5 mmol), and HMPA (0.5 g) in xylene (20 mL) was allowed to reflux for 12 h. The mixture was made acidic with 10% HCl. The resulting precipitates were collected and recrystallized from CH_2Cl_2 to give α,α-bis(7-hydroxy-5-isopropyltropon-2-yl)-3,4-(methylenedioxy)toluene (**4m**; 2.9 g, 41%): mp 214–215 °C; NMR ($CDCl_3$) δ 5.99 (2 H, s, OCH_2O), 6.61 (1 H, s, CH); MS, m/e 460 (M^+). Anal. ($C_{28}H_{28}O_6$) C, H.

α,α-Bis(7-hydroxy-5-isopropyltropon-2-yl)toluene (4d). Method C. Compound **4c** (1 g, 2 mmol) was hydrogenated in dioxane–EtOH in the presence of 10% Pd–carbon (0.1 g). The residue, obtained by workup in the usual way, was chromatographed on silica gel with hexane–AcOEt (4:1) to give **4d**: 0.76 g (91%); mp 219–220 °C (from MeOH–CH_2Cl_2); NMR (Me_2SO-d_6) δ 6.61 (1 H, s, CH), 8.50–9.50 (3 H, br s, 3 OH); MS, m/e 432 (M^+). Anal. ($C_{27}H_{28}O_5$) C, H.

2-Amino-α,α-bis(7-hydroxy-5-isopropyltropon-2-yl)toluene (4f). Method D. A mixture of 4-acetamino-α,α-bis(7-hydroxy-5-isopropyltropon-2-yl)toluene (**4e**; 2.2 g, 5 mmol), acetic acid (30 mL), and 10% HCl (40 mL) was allowed to reflux for 10 h, poured into ice water, made basic with saturated $KHCO_3$ solution, and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated. Recrystallization of the resulting precipitates from MeOH–CH_2Cl_2 gave **4f**: 1.3 g (66%); mp 244–245 °C. Anal. ($C_{27}H_{29}NO_4$) C, H.

4-[Bis(7-hydroxy-5-isopropyltropon-2-yl)methyl]phenyltrimethylammonium Iodide (4g). A solution of α,α-bis(7-hydroxy-5-isopropyltropon-2-yl)-4-(dimethylamino)toluene[2] (1.6 g, 1 mmol) and methyl iodide (4.2 g, 8 mmol) in CH_2Cl_2 (30 mL) was stirred for 2 days at room temperature. After the solvent was evaporated, the residue was recrystallized from MeOH–CH_2Cl_2 to give **4g**: 2 g (95%); mp 143–145 °C; NMR (Me_2SO-d_6) δ 3.98 (9 H, s, NMe_3), 6.70 (1 H, s, OH).

α,α-Bis(7-hydroxy-5-isopropyltropon-2-yl)-4-carboxytoluene (4i). Method E. A solution of **4h** (0.8 g, 2 mmol) of NaOH (10 g) in MeOH–H_2O (100 mL) was allowed to reflux for 20 min, and then the MeOH was evaporated. The aqueous solution that remained was made acidic with 5% HCl and extracted with CH_2Cl_2. The CH_2Cl_2 layer was dried over $MgSO_4$ and concentrated. The resulting precipitates were recrystallized from Et_2O–CH_2Cl_2 to give **4i**: 0.6 g (82%); mp 256–257 °C; NMR ($CDCl_3$) δ 6.78 (1 H, s, CH), 8.20 (2 H, d, J = 8 Hz, 3-H and 5-H), 10.15 (3 H, s, 3 OH); MS, m/e 460 (M^+). Anal. ($C_{28}H_{28}O_6$) C, H.

Similarly, [4-[bis(7-hydroxy-5-isopropyltropon-2-yl)methyl]-phenoxy]acetic acid was prepared from ethyl [4-[bis(7-hydroxy-5-isopropyltropon-2-yl)methyl]phenoxy]acetate (**4j**) in 73% yield and converted to its sodium salt (**4k**) by the treatment with equimolar 10% NaOH solution.

X-ray Structural Analysis. Intensity measurements were made on a Rigaku four-circle diffractomer using graphite-monochromated Cu Kα (1.5418 Å) radiation. The structure was solved by the direct methods computer program MULTAN. Several refinement circles led to an R value (not including hydrogen atoms) of 0.096.

Biological Assays. Assays of antitumor activity were carried out according to methods described previously.[3]

Registry No. **1**, 499-44-5; **2a**, 774-48-1; **2b**, 2403-58-9; **2c**, 33210-81-0; **2e**, 101403-68-3; **2h**, 101403-69-4; **2j**, 101403-70-7; **2l**, 15222-63-6; **2m**, 40527-42-2; **2n**, 101403-71-8; **2o**, 33224-62-3; **2p**, 13959-97-2; **2q**, 13529-27-6; **2r**, 101403-72-9; **4** (R = NMe_2), 101403-76-3; **4** (R = OCH_2CO_2H), 101403-77-4; **4a**, 101403-50-3; **4b**, 101403-51-4; **4c**, 101403-52-5; **4d**, 101403-53-6; **4e**, 101403-54-7; **4f**, 101403-55-8; **4g**, 101403-56-9; **4h**, 101403-57-0; **4i**, 101403-58-1; **4j**, 101403-59-2; **4k**, 101403-60-5; **4l**, 101403-61-6; **4m**, 101403-62-7; **4n**, 101403-63-8; **4o**, 101403-64-9; **4p**, 101403-65-0; **4q**, 101403-66-1; **4r**, 101403-67-2; **5b**, 101403-73-0; **5c**, 101403-74-1; **5o**, 101403-75-2.

Supplementary Material Available: Listings of X-ray data, bond lengths, bond angles, and final atomic positional parameters and thermal parameters for **4b** (4 pages). Ordering information is given on any current masthead page.