

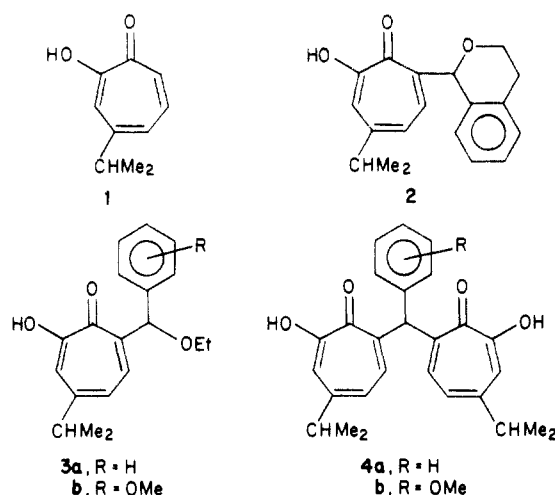
## Synthesis and Antitumor Activity of Tropolone Derivatives. 2

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Structural requirement for antitumor activity of tropolone derivatives 2-4 was explored. Isochroman derivatives (6-17, 20, and 23) and  $\alpha,\alpha$ -disubstituted compounds 26-30 were synthesized and their antitumor activities were tested. These nontropolone derivatives were all inactive, implying that a tropolone ring is essential for the activity. Several compounds related to the monotropolone analogue 3 were synthesized. Among them, 31-33 showed significant activity, but their potencies were considerably weaker than those of binary tropolone analogues 4.

In previous papers,<sup>1,2</sup> we have reported the syntheses of new tropolone derivatives and their antitumor activities. Typical antitumor compounds are 3-(isochroman-1-yl)-6-isopropyltropolone (2), 3-( $\alpha$ -ethoxybenzyl)-6-isopropyltropolones (3), and  $\alpha,\alpha$ -bis(2-hydroxy-6-isopropyltropolon-3-yl)toluenes (4), which inhibit the growth of KB cells (in vitro system) and which are active in the survival test by use of P388 mice (in vivo system). These compounds (2-4) possess, without exception, a hinokitiol<sup>3</sup> moiety, which is a constituent of the plants of *Chamaecyparis* species.



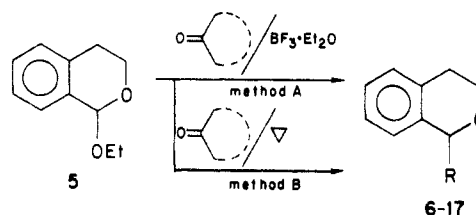
As the hinokitiol moiety is, obviously, essential to the antitumor activity, the aim of our initial effort was to develop some other effective groups that can substitute for tropolone ring in the antitumor action. Isochroman derivatives 6-17, 20, and 23 containing various carbonyl components or heterocyclic rings and  $\alpha,\alpha$ -disubstituted toluene analogues 26-30 were synthesized and their antitumor activities were tested. Consequently, these nontropolone derivatives were all inactive even in the in vitro system, implying that the presence of a tropolone ring in a molecule is essential to the activity. Therefore, our attention was focused on the molecular modification of moderately active monotropolones 3.

This paper describes the syntheses of compounds related to 2, 3, or 4 and their antitumor activities.

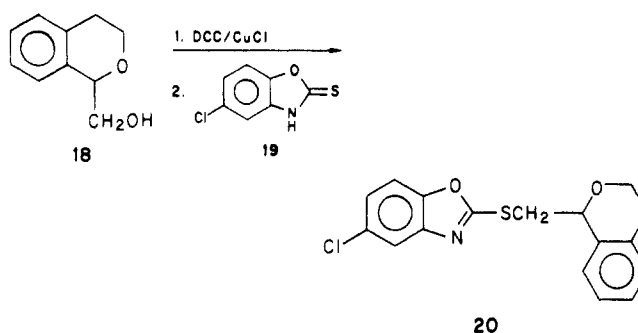
**Chemistry.** Isochromanyl derivatives 6-16 were prepared by the reaction of 1-ethoxyisochroman (5) with the corresponding active methylene compounds, either in the presence of boron trifluoride-diethyl etherate at about 30 °C (method A) or in refluxing xylene (method B).<sup>4-6</sup>

Benzoxazole derivatives 17, 20, and 23 were prepared by application of our finding<sup>7</sup> obtained from the study on the alkylation of benzoxazole derivatives. A mixture of

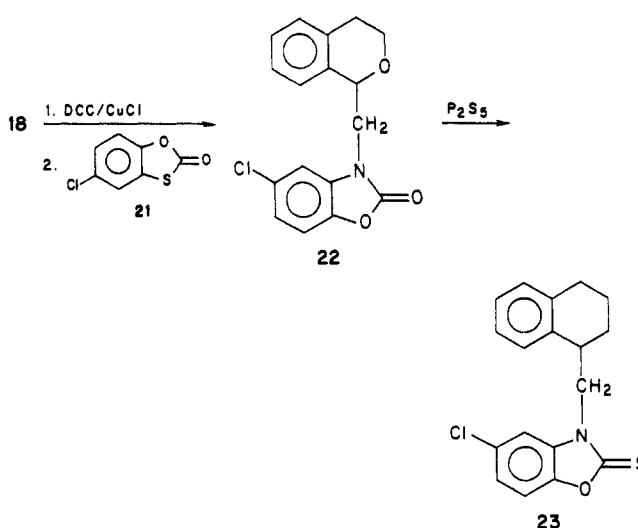
Scheme I



Scheme II. Method C



Scheme III. Method D



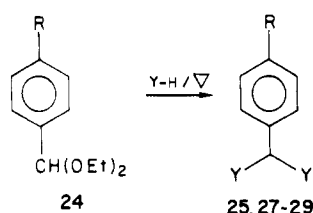
5 and 5-chlorobenzoxazoline-2-thione<sup>8</sup> (19) was heated to give 3-(isochroman-1-yl)benzoxazoline-2-thione (17) (me-

<sup>†</sup> Okayama University.

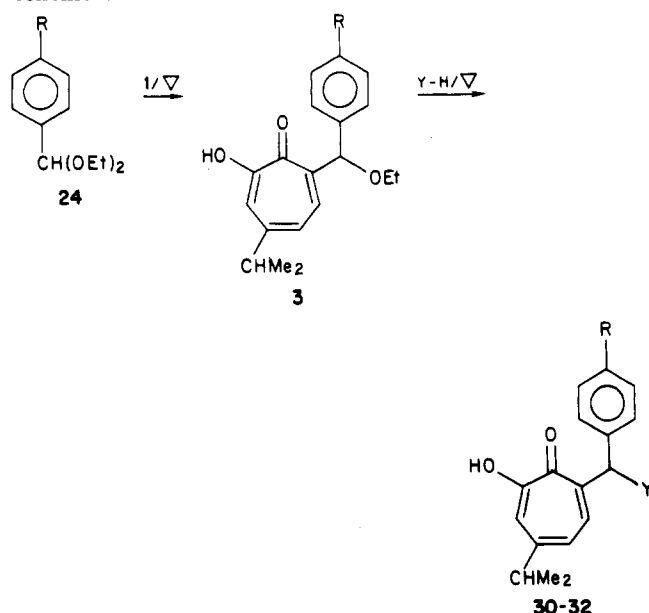
<sup>‡</sup> Cancer Chemotherapy Center.

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Scheme IV. Method E



Scheme V. Method F



thod B). Treatment of (isochroman-1-yl)methanol (18) with *N,N'*-dicyclohexylcarbodiimide (DCC) afforded the corresponding alkoxyisourea, which was heated without purification with 19 at 150 °C to give 2-[(isochroman-1-ylmethyl)thio]benzoxazole (20) (method C). Similarly, treatment of 18 with DCC followed by the reaction of 5-chlorobenzoxazolin-2-one<sup>9</sup> (21) gave 3-(isochroman-1-ylmethyl)benzoxazolin-2-one (22), which was converted to a thiocarbonyl analogue 23 by the treatment with phosphorus pentasulfide (method D).

Binary compounds (25 and 27-29) were prepared by heating of benzaldehyde diethyl acetals (24) with the corresponding nucleophiles (method E). For example, a mixture of *p*-methoxybenzaldehyde diethyl acetal (24a) and kojic acid was heated at 140 °C for 2.5 h to give  $\alpha,\alpha$ -bis[2-hydroxy-1-(hydroxymethyl)-4-oxo-4*H*-pyran-2-yl]-4-methoxytoluene (29) in a 75% yield.

3-( $\alpha$ -Ethoxy-4-methoxybenzyl)-6-isopropyltropolone (3a),<sup>1</sup> prepared from benzaldehyde diethyl acetal (24a) and hinokitiol (1), was heated with acetamide to give  $\alpha$ -acetamino derivative 30 (method F). Similarly, 31 and 32 were prepared by heating of 3a with benzamide or benzoxazoline-2-thione, respectively. Benzaldehyde diphenyl thioacetal was heated with hinokitiol (1) at 160 °C for 7

h to give 3-[ $\alpha$ -(phenylthio)-4-methoxybenzyl]-6-isopropyltropolone (33) in a 34% yield.

## Biological Results and Discussion

Compounds listed in Tables I-III were evaluated for inhibition of growth of KB cells<sup>10</sup> (in vitro) and antitumor activity against leukemia P388 in mice (in vivo).

As previously reported,<sup>1</sup> methylation of the hydroxyl group of the tropolone ring of 2 resulted in loss of the antitumor activity even in the in vitro system. This fact suggested that the presence of an acidic hydroxyl group might play an important role in the antitumor action and led us to synthesize isochroman-1-ylphenols 6-9 (Table I). However, they were all inactive in the in vitro system. Compounds 10-14 having a carbonyl and/or an acidic hydroxyl group in analogy with hinokitiol derivatives were also to be inactive. Compounds 15-17, 20, and 23 contain a heterocyclic ring such as uracil, tenuazoic acid,<sup>11</sup> or 2-thiobenzoxazole,<sup>12</sup> which is a component of known antitumor compounds. However, they showed no significant antitumor activity.

We previously found that the activities of binary tropolone analogues 4 were about 200 times those of mono-tropolone analogues 3, almost comparable to that of colchicine in the in vivo system.<sup>1</sup> However, the reason why two tropolone rings are necessary to exhibit potent activity in the in vivo system remains unknown. Presently, we synthesized binary type of compounds related to the tropolones 4 in order to clarify the above reason. Namely, 26 and 27 have two aromatic rings that contain hydroxyl and isopropyl groups in analogy with hinokitiol. The remaining binary compounds 28-30 were designed to have an acidic hydroxyl and proton-acceptable groups such as methoxy or carbonyl groups situated in the near position which permit hydrogen-bond formation. Contrary to expectation, they were also inactive.

The observation that all nontropolone derivatives were inactive showed that a tropolone ring was essential to the activity both in vitro and in vivo. Consequently, we have modified monotropolone analogues 3. Substitution of the ethoxy group of 3a with an acetamino or benzoylamino group, which is necessary for antitumor activity of colchicines,<sup>13</sup> gave  $\alpha$ -acetamino (30) and  $\alpha$ -benzoylamino (31) analogues, respectively. Compound 30 was inactive, while 31 exhibited a significant activity in the in vitro and in vivo systems. Compound 32, which is  $\alpha$ -(2-hydroxy-6-isopropyltropolon-3-yl)- $\alpha$ -substituted toluene, a so-called binary compound, by two different groups, was active in the in vivo system. But the activity of 32 was considerably weaker than those of the binary tropolone analogues 4.

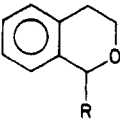
In summary, several new active compounds were prepared; however, analogues that are more active than binary tropolone derivatives 4 could not be developed.



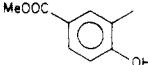
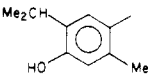
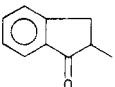
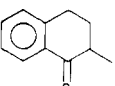
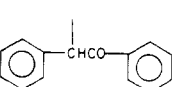
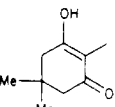
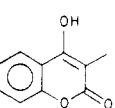
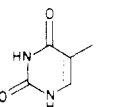
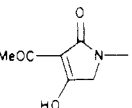
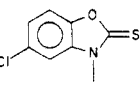
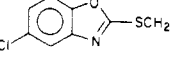
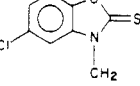
## Experimental Section

Melting points are uncorrected. NMR spectra were run on a Hitachi R-24 spectrometer at 60 MHz, with  $\text{Me}_4\text{Si}$  as an internal standard. Mass spectra were taken with Shimadzu LKB 9000 spectrometer. The elemental analyses were within 0.4% of the

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**Table I.** Antitumor Activity of Isochromanyl Derivatives


compd	R	ref or <sup>a</sup> method	crystn solvent	mp, °C	yield, %	formula	inhibn of KB cell <sup>b</sup> growth, ID <sub>50</sub>	
							μg/mL	nM/mL
6		4	benzene	113–115	22	C <sub>15</sub> H <sub>14</sub> O <sub>2</sub>	27	119
7		4	MeOH	150–151	21	C <sub>15</sub> H <sub>14</sub> O <sub>2</sub>	20.5	90
8		4	CH <sub>2</sub> Cl <sub>2</sub>	142–144	41	C <sub>17</sub> H <sub>16</sub> O <sub>4</sub>	>100	352
9		A	oil		66	C <sub>19</sub> H <sub>22</sub> O <sub>2</sub>	16.0	57
10		A	Et <sub>2</sub> O	134–136	53	C <sub>18</sub> H <sub>16</sub> O <sub>2</sub>	38.0	144
11		5	Et <sub>2</sub> O	123–124	59	C <sub>19</sub> H <sub>18</sub> O <sub>2</sub>	42.0	151
12		A	Et <sub>2</sub> O	138–141	50	C <sub>23</sub> H <sub>20</sub> O <sub>2</sub>	>100	305
13		5	Et <sub>2</sub> O	120–121	80	C <sub>17</sub> H <sub>20</sub> O <sub>3</sub>	>100	368
14		A	MeOH	167–171	71	C <sub>18</sub> H <sub>14</sub> O <sub>4</sub>	100	340
15		6	THF	303–304	61	C <sub>13</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub>	>100	410
16		B	AcOEt–PE <sup>c</sup>	77–79	90	C <sub>15</sub> H <sub>16</sub> NO <sub>4</sub>	>100	366
17		B	cyclohexane	140–141	64	C <sub>16</sub> H <sub>12</sub> ClNO <sub>2</sub> S	85	268
20		C	AcOEt–PE	95–96	<i>d</i>	C <sub>17</sub> H <sub>14</sub> ClNO <sub>2</sub> S	6.1	18
23		D	benzene	151–152	<i>d</i>	C <sub>17</sub> H <sub>14</sub> ClNO <sub>3</sub>	8.9	28

<sup>a</sup>The Arabic numerals show reference. <sup>b</sup>The compounds listed are inactive in the survival test of P388 mice at 400 mg/kg. <sup>c</sup>PE = petroleum ether. <sup>d</sup>See Experimental Section.

theoretical values. Column chromatographic separation were performed by flash technique.

**Method A. 2-Isochroman-1-yl-1,2-dihydroinden-1-one (10).** BF<sub>3</sub>·Et<sub>2</sub>O (47%, 4 mL) was added dropwise to a solution of 1-ethoxyisochroman (5; 20 g, 112 mmol) and 1,2-dihydroinden-1-one (10 g, 76 mmol) in dry benzene (50 mL). The mixture was stirred at 30–40 °C for 3 h, diluted with Et<sub>2</sub>O (100 mL), and washed with H<sub>2</sub>O. After the solvent was removed, the residue was crystallized from Et<sub>2</sub>O to give 10 (10.6 g, 53%): mp 134–136 °C; IR (Nujol) 1710 cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>) δ 2.3–3.6 (m, 5 H, 2-H, 3-H<sub>2</sub>, 4'-H<sub>2</sub>),

3.6–4.3 (m, 2 H, 3'-H<sub>2</sub>), 5.58 (d, 1 H, 1'-H, *J* = 2 Hz), 7.0–8.0 (m, 8 H, aromatic H); MS, *m/z* 264 (M<sup>+</sup>). Anal. (C<sub>18</sub>H<sub>16</sub>O<sub>2</sub>) C, H.

**Method B. 5-Chloro-3-isochroman-1-ylbenzoxazolin-2-thione (17).** A solution of 5 (4 g, 23 mmol) and 5-chlorobenzoxazolin-2-thione<sup>8</sup> (19; 3.1 g, 17 mmol) in dry xylene (200 mL) was refluxed for 5 h, washed with H<sub>2</sub>O, and concentrated. The residue was crystallized from cyclohexane to give 17 (3.4 g, 64%): mp 140–141 °C; UV (CHCl<sub>3</sub>) λ<sub>max</sub> 314 nm (ε 30199), 268 (10232), 262 (10715); NMR (CDCl<sub>3</sub>) δ 2.6–3.6 (m, 2 H, 4'-H<sub>2</sub>), 3.9–4.5 (m, 2 H, 3'-H<sub>2</sub>), 6.51 (d, 1 H, 4-H, *J* = 2 Hz), 6.9–7.6 (m, 7 H, 1-H

Table II. Antitumor Activity of Binary Compounds

compd	R	Y	inhibn of KB cell growth, ID <sub>50</sub>		antitumor act. P388 in mice, ip <sup>a</sup>	
			μg/mL	nM/mL	doses, mg/kg	T/C, %
4a <sup>b</sup>	H		0.3	0.7	5	188
					2.5	150
					1.3	132
4b <sup>b</sup>	OMe		0.5	1.1	5	173
					2.5	134
					0.6	127
25	H		13	34	400	102
					200	100
					100	87
26	COMe		55	107	400	88
					200	89
					100	91
27	H		55	132	400	83
					200	100
					100	91
28	H		76	163	50	97
					25	95
					12	103
29	OMe		100	249	200	102
					100	98
					50	102

<sup>a</sup> The doses listed was given once a day for 1 and 5 days. <sup>b</sup> See ref 1.

and aromatic H); MS,  $m/z$  319 ( $M^+ + 2$ ), 317 ( $M^+$ ). Anal. ( $C_{10}H_{12}ClNO_2S$ ) C, H, N.

**Isochroman-1-ylmethanol (18).** A mixture of 1-isochromancarboxylic acid<sup>14</sup> (15 g, 84 mmol),  $LiAlH_4$  (4.8 g, 127 mmol), and  $Et_2O$  (150 mL) was refluxed for 1 h. The reaction mixture was worked up in the usual way to give 18 (9.6 g, 70%) as an oil: bp 113–114 °C (1 mmHg); NMR ( $CDCl_3$ )  $\delta$  2.6–3.0 (m, 2 H, 4'-H<sub>2</sub>), 3.01 (s, 1 H, OH), 3.5–4.0 (m, 4 H, 3'-H<sub>2</sub>, CH<sub>2</sub>OH), 4.88 (dd, 1 H, 1'-H,  $J = 5, 7$  Hz), 7.17 (s with shoulder, 4 H, aromatic H); MS,  $m/z$  164 ( $M^+$ ). Anal. ( $C_{10}H_{12}O_2$ ) C, H.

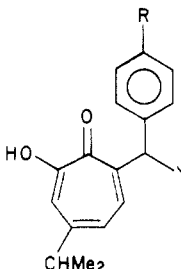
**Method C. 5-Chloro-2-[(isochroman-1-ylmethyl)thio]benzoxazole (20).** A mixture of 18 (1.8 g, 11 mmol),  $N,N'$ -dicyclohexylcarbodiimide (DCC; 2.5 g, 12 mmol), CuCl (catalytic amount), and tetrahydrofuran (THF; 100 mL) was stirred at room temperature for 12 h and 5-chlorobenzoxazolin-2-thione<sup>8</sup> (19; 2.1 g, 11 mmol) was added to the mixture. The mixture was refluxed for 6 h and the  $N,N'$ -dicyclohexylurea formed was filtered off. The filtrate was concentrated and the residue was chromatographed on alumina (Wako, 200–300 mesh) with petroleum ether–AcOEt (4:1) to give 20 (1.8 g, 50%): mp 94.5–95.5 °C; NMR ( $Me_2CO-d_6$ )  $\delta$  2.6–3.1 (m, 2 H, 4'-H<sub>2</sub>), 3.3–4.3 (m, 4 H, 3'-H<sub>2</sub>, SCH<sub>2</sub>),

5.15 (dd, 1 H, 1'-H,  $J = 3.5, 8.5$  Hz), 7.0–7.6 (m, 7 H, aromatic H); MS,  $m/z$  333 ( $M^+ + 2$ ), 331 ( $M^+$ ). Anal. ( $C_{17}H_{14}ClNO_2S$ ) C, H, N.

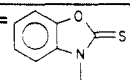
**Method D. 5-Chloro-3-(isochroman-1-ylmethyl)benzoxazolin-2-thione (23).** A mixture of 18 (3.8 g, 23 mmol), DCC (5 g, 24 mmol), CuCl (catalytic amount), and THF (100 mL) was stirred at room temperature for 12 h and concentrated in vacuo. To the residue, 5-chlorobenzoxazolin-2-one<sup>9</sup> (21; 3.9 g, 23 mmol) was added. The mixture was heated at 150 °C for 2.5 h and diluted with THF (20 mL). The  $N,N'$ -dicyclohexylurea formed was filtered off and the filtrate was concentrated. The residue was chromatographed on silica gel (Wako C-300, 200–300 mesh) with petroleum ether–AcOEt (5:1) to give 5-chloro-3-(isochroman-1-ylmethyl)benzoxazolin-2-one (22; 3.1 g, 44%): mp 150–152 °C; IR (Nujol) 1760  $cm^{-1}$ ; NMR ( $CDCl_3$ )  $\delta$  2.7–3.0 (2 H, m, 4'-H<sub>2</sub>), 3.5–4.5 (m, 4 H, 3'-H<sub>2</sub>, NCH<sub>2</sub>), 5.18 (dd, 1 H, 1'-H,  $J = 2, 8$  Hz), 7.1–7.5 (m, 7 H, aromatic H); MS,  $m/z$  317 ( $M^+ + 2$ ), 315 ( $M^+$ ). Anal. ( $C_{17}H_{14}ClNO_3$ ) C, H, N.

A suspension of 22 (2.8 g, 9 mmol) and  $P_2S_5$  (5.5 g, 25 mmol) in xylene (100 mL) was refluxed for 9 h and the hot reaction mixture was filtered. To the filtrate was added  $Et_2O$  and the  $Et_2O$  solution was washed with concentrated  $NH_3$  solution. After the solvent was removed, the residue was crystallized from benzene to give 23 (2.3 g, 78%): mp 151–152 °C; MS,  $m/z$  333 ( $M^+ + 2$ ), 331 ( $M^+$ ). Anal. ( $C_{17}H_{14}ClNO_2S$ ) C, H, N.

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**Table III.** Antitumor Activity of Monotropolone Derivatives


compd	R	Y	inhibn of KB cell growth, ID <sub>50</sub>		antitumor act. P388 in mice, ip <sup>a</sup>	
			μg/mL	nM/mL	doses, mg/kg	T/C, %
3a <sup>b</sup>	H	OEt	0.3	1.0	400	0
					200	109
					100	102
					50	97
					25	96
3b <sup>b</sup>	OMe	OEt	0.5	1.5	12.5	96
					400	140
					200	128
					100	140
					50	119
30	OMe	NHCOMe	6.0	18.0	25	109
					12.5	96
					400	108
31	OMe	NHCOC <sub>6</sub> H <sub>5</sub>	1.7	4.2	200	92
					100	92
					400	131
32	OMe	Boz <sup>c</sup>	3.7	8.5	200	110
					100	104
					400	133
33	H	SC <sub>6</sub> H <sub>5</sub>	0.5	1.4	200	105
					100	113
					400	113
					200	135
					100	108

<sup>a</sup> The doses listed were given once a day for 1 and 5 days. <sup>b</sup> See ref 1. <sup>c</sup> Boz = .

**α,α-Bis(4-hydroxy-5-isopropyl-2-methoxyphenyl)toluene (25).** BF<sub>3</sub>·Et<sub>2</sub>O (47%, 3 drops) was added to a mixture of benzaldehyde diethyl acetal (**24a**; 12 g, 66 mmol) and thymol (15 g, 100 mmol) at 0 °C and the mixture was stirred at 0–5 °C for 2 h. The reaction mixture was warmed to 80–100 °C until precipitates separated out. They were again dissolved and the mixture allowed to stand at room temperature overnight. The resulting precipitate was collected with suction, washed successively with dilute HCl solution and H<sub>2</sub>O, and recrystallized from benzene to give **25** (14.5 g, 75%): mp 169–172 °C; NMR (CDCl<sub>3</sub>) δ 1.08 (d, 12 H, Me × 4, *J* = 7 Hz), 2.05 (s, 6 H, Me × 2), 2.8–3.4 (m, 2 H, CHMe<sub>2</sub> × 2), 4.60 (s, 2 H, OH), 5.57 (s, 1 H, α-H), 6.62 (s, 4 H, aromatic H), 6.9–7.5 (m, 5 H, aromatic H); MS, *m/z* 388 (M<sup>+</sup>). Anal. (C<sub>27</sub>H<sub>32</sub>O<sub>2</sub>) C, H.

**α,α-Bis(4-acetoxy-5-propyl-2-methylphenyl)-4-acetyl-toluene (26).** To a solution of AlCl<sub>3</sub> (1.1 g, 8.2 mmol) in dry CS<sub>2</sub> (20 mL) was added a solution of **27** (3 g, 7.7 mmol) and acetyl chloride (0.65 g, 8.3 mmol) in CS<sub>2</sub> (50 mL) at 0–5 °C and the mixture was stirred at 0–5 °C for 3 h. The reaction mixture was stirred at room temperature overnight, concentrated, decomposed with dilute HCl solution, and extracted with Et<sub>2</sub>O. After the solvent was removed, the residue was chromatographed on silica gel with petroleum ether–AcOEt (10:1) to give **26** (0.84 g, 21%): mp 146–148 °C; IR (Nujol) 1760, 1690 cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>) δ 1.00 (d, 12 H, CHMe<sub>2</sub> × 2, *J* = 7 Hz), 2.10 (s, 6 H, Me × 2), 2.30 (s, 6 H, OCOMe × 2), 2.60 (s, 3 H, COMe), 5.73 (s, 1 H, α-H), 6.70 (s, 2 H, aromatic H), 6.89 (s, 2 H, aromatic H), 7.18 (d, 2 H, aromatic H, *J* = 8 Hz), 7.96 (d, 2 H, aromatic H, *J* = 8 Hz); MS, *m/z* 514 (M<sup>+</sup>). Anal. (C<sub>33</sub>H<sub>38</sub>O<sub>5</sub>) C, H.

**Method E. α,α-Bis[3-hydroxy-6-(hydroxymethyl)-4-oxo-4H-pyran-2-yl]-4-methoxytoluene (29).** A mixture of *p*-anis-

aldehyde diethyl acetal (**24b**; 0.74 g, 1.2 mmol) and kojic acid (1 g, 2.5 mmol) was heated at 140 °C for 3 h. The resulting precipitate was filtered off and recrystallized from MeOH to give **29** (1.06 g, 75%): mp 248–250 °C; IR (Nujol) 3300, 1660, 1620 cm<sup>-1</sup>; NMR (Me<sub>2</sub>SO-*d*<sub>6</sub>) δ 3.72 (s, 3 H, OMe), 4.31 (s, 4 H, CH<sub>2</sub>OH × 2), 5.25 (br s, 2 H, CH<sub>2</sub>OH × 2), 6.10 (s, 1 H, α-H), 6.40 (s, 2 H, 3'-H × 2), 6.93 (d, 2 H, 3-H, 5-H, *J* = 9 Hz), 7.33 (d, 2 H, 2-H, 6-H, *J* = 9 Hz), 8.7–9.7 (br s, 2 H, OH × 2). Anal. (C<sub>20</sub>H<sub>18</sub>O<sub>9</sub>) C, H.

Compounds **27** and **28** were prepared in the same manner.

**α,α-Bis(5-allyl-2-hydroxy-3-methoxyphenyl)toluene (27):** mp 144–146 °C (from Et<sub>2</sub>O); yield 23%; NMR (CDCl<sub>3</sub>) δ 3.27 (d, 4 H, CH<sub>2</sub>CH=CH<sub>2</sub> × 2, *J* = 6 Hz), 3.76 (s, 6 H, OMe × 2), 4.7–5.3 (m, 4 H, CH<sub>2</sub>CH=CH<sub>2</sub> × 2), 5.69 (s, 2 H, OH × 2), 5.7–6.2 (m, 2 H, CH<sub>2</sub>CH=CH<sub>2</sub> × 2), 6.26 (s, 1 H, α-H), 6.46 (s, 2 H, aromatic H), 6.67 (s, 2 H, aromatic H), 7.27 (s, 5 H, C<sub>6</sub>H<sub>5</sub>); MS, *m/z* 416 (M<sup>+</sup>).

**2,2'-Benzylidenebis(3-hydroxy-1,4-naphthoquinone) (28):** mp 232–234 °C (from EtOH–dioxane); yield 83%; NMR (Me<sub>2</sub>SO-*d*<sub>6</sub>) δ 3.70 (s, 3 H, OMe), 5.4 (br s, 2 H, OH × 2), 6.00 (s, 1 H, α-H), 6.71 (d, 2 H, aromatic H, *J* = 8 Hz), 7.20 (d, 2 H, aromatic H, *J* = 8 Hz), 7.6–8.2 (m, 8 H, aromatic H); MS, *m/z* 466 (M<sup>+</sup>). Anal. (C<sub>28</sub>H<sub>18</sub>O<sub>7</sub>) C, H.

**Method F. 3-[α-(Acetylamino)-4-methoxybenzyl]-6-isopropyltropolone (30).** A mixture of 3-(α-ethoxy-4-methoxybenzyl)-6-isopropyltropolone<sup>2</sup> (**3b**; 0.9 g, 2.7 mmol) and acetamide (0.19 g, 3.3 mmol) was heated at 160 °C for 3 h under an argon atmosphere. The residue was crystallized from pyridine to give **30** (0.49 g, 52%): mp 246–247 °C; IR (Nujol) 3150, 1620 cm<sup>-1</sup>; NMR (pyridine-*d*<sub>5</sub>) δ 1.15 (d, 6 H, CHMe<sub>2</sub>, *J* = Hz), 2.15 (s, 3 H, COMe), 2.5–3.2 (m, 1 H, CHMe<sub>2</sub>), 3.64 (s, 3 H, OMe), 6.7–8.2 (m,

9 H,  $\alpha$ -H, aromatic H, tropolone H, NH), 9.2 (br s, 1 H, OH). Anal. ( $C_{20}H_{23}NO_4$ ) C, H, N.

Compounds 31 and 32 were prepared in the same manner.

**3-[( $\alpha$ -(Benzoylamino)-4-methoxybenzyl]-6-isopropyl-tropolone (31):** mp 172–173 °C (from  $CH_2Cl_2$ -MeOH); yield 53%; NMR ( $CDCl_3$ )  $\delta$  1.31 (d, 6 H,  $CHMe_2$ ,  $J = 7$  Hz), 2.5–3.3 (m, 1 H,  $CHMe_2$ ), 3.79 (s, 3 H, OMe), 6.4–8.2 (m, 12 H,  $\alpha$ -H, aromatic H, tropolone H, NH), 8.60 (d, 2 H, aromatic H,  $J = 9$  Hz), 8.2–8.8 (br s, 1 H, OH). Anal. ( $C_{26}H_{25}NO_4$ ) C, H, N.

**3-[( $\alpha$ -(2-Hydroxy-6-isopropyltropo-3-yl)-4-methoxybenzyl]benzoxazoline-2-thione (32):** mp 163–166 °C (from  $CH_2Cl_2$ -MeOH); yield 41%; IR (Nujol) 3150; NMR ( $CDCl_3$ )  $\delta$  1.30 (d, 6 H,  $CHMe_2$ ,  $J = 7$  Hz), 2.5–3.2 (m, 1 H,  $CHMe_2$ ), 3.81 (s, 3 H, OMe), 6.7–8.0 (m, 1 H, aromatic H), 8.3–9.3 (br, 1 H, OH); MS,  $m/z$  433 ( $M^+$ ). Anal. ( $C_{25}H_{23}NO_4S$ ) C, H, N.

**3-[( $\alpha$ -(Phenylthio)benzyl]-6-isopropyltropolone (33).** A mixture of hinokitiol (1; 2 g, 12.2 mmol) and benzaldehyde diphenyl thioacetal (6.0 g, 19.5 mmol) was heated at 160 °C for 7 h under an argon atmosphere. The reaction mixture was chromatographed on silica gel with petroleum ether-AcOEt (25:1) to give 33 (1.5 g, 34%): mp 105–106 °C (from MeOH); IR (Nujol) 3200  $cm^{-1}$ ; NMR ( $CDCl_3$ )  $\delta$  1.28 (d, 6 H,  $CHMe_2$ ,  $J = 7$  Hz), 2.6–3.2

(m, 1 H,  $CHMe_2$ ), 6.58 (s, 1 H,  $\alpha$ -H), 6.8–8.2 (m, 13 H, aromatic H, tropolone H), 8.5–9.5 (br, 1 H, OH); MS,  $m/z$  362 ( $M^+$ ). Anal. ( $C_{23}H_{22}O_2S$ ) C, H.

**Biological Assays.** Assays of antitumor activity were carried out as described previously.<sup>1</sup>

**Registry No.** 1, 499-44-5; 3b, 96292-82-9; 5, 75802-22-1; 6, 77317-00-1; 7, 77316-99-5; 8, 96292-83-0; 9, 96292-84-1; 10, 96292-85-2; 11, 82584-05-2; 12, 96292-86-3; 13, 96292-87-4; 14, 96292-88-5; 15, 96292-89-6; 16, 96292-90-9; 17, 96292-91-0; 18, 96292-92-1; 19, 22876-19-3; 20, 96292-93-2; 21, 95-25-0; 22, 96292-94-3; 23, 96292-95-4; 24a, 774-48-1; 24b, 2403-58-9; 25, 96292-96-5; 26, 96292-97-6; 27, 96292-98-7; 28, 96306-50-2; 29, 96306-51-3; 30, 96292-99-8; 31, 96293-00-4; 32, 96293-01-5; 33, 96293-02-6;  $B_3NH_2$ , 55-21-0; Boz-H, 2382-96-9; 1,2-dihydroinden-1-one, 480-90-0; 1-isochromancarboxylic acid, 13328-85-3; thymol, 89-83-8; kojic acid, 501-30-4; 2-methoxy-4-allylphenol, 97-53-0; 2-hydroxy-1,4-naphthalenedione, 83-72-7; acetamide, 60-35-5; benzaldehyde diphenyl dithioacetal, 7695-69-4; 5-methyl-2-isopropylphenol, 89-83-8; benzylcarbonylbenzene, 451-40-1; 4-hydroxy-1-benzopyran-2-one, 1076-38-6; 3-acetyl-4-hydroxy-3-pyrrolin-2-one, 2113-93-1.

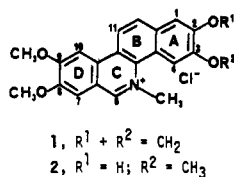
## Synthesis and Antitumor Activity of Structural Analogues of the Anticancer Benzophenanthridine Alkaloid Fagaronine Chloride

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The indenoisoquinoline analogue 4 of fagaronine chloride (2) has been prepared, as well as its positional isomer 20 and the corresponding mesylated derivatives 16 and 19. Compounds 4, 16, and 20 were tested against P388 lymphocytic leukemia and found to possess significant activity. A tricyclic analogue 24 was also synthesized and was devoid of cytotoxicity in the KB cancer cell culture system. The change in the substitution pattern of the A-ring on going from 4 to 20 was tolerated without producing a significant decrease in antitumor activity.

The benzo[c]phenanthridine alkaloids nitidine (1) and fagaronine (2) have been isolated from *Zanthoxylum ni-*



*tidium*<sup>1,2</sup> and *Fagara zanthoxyloides*,<sup>3</sup> respectively. The structure elucidation of nitidine (1) involved its chemical conversion to known compounds<sup>2</sup> as well as the synthesis of dihydronitidine,<sup>4</sup> while that of fagaronine (2) was proposed after NMR analysis of its *N*-demethyl derivative<sup>5</sup>

**Table I.** Evaluation of the Indenoisoquinoline Analogue 3 of Nitidine (1) for Anticancer Activity in the M5076 Sarcoma System<sup>a</sup>

compd	dose, mg/kg	survival	wt diff	% T/C
3	400	0/10		
	200	6/10	-7.2	
	100	9/9	-4.4	137
	50	9/9	-2.0	107
	25	10/10	-0.3	113
	12.5	10/10	-0.2	109
	6.25	10/10	-0.8	115

<sup>a</sup> For the general screening procedure and data interpretation, see ref 19.

and confirmed by total synthesis.<sup>6</sup> Nitidine (1) has also been synthesized by several methods.<sup>4,7</sup>

Recent interest in nitidine (1) and fagaronine (2) has been stimulated by their activity against the mouse Leu-

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