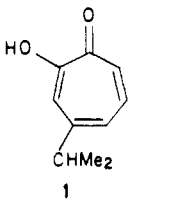
Synthesis and Antitumor Activity of Tropolone Derivatives. 2

Masatoshi Yamato,*† Kuniko Hashigaki,† Shigetaka Ishikawa,† Nobuhiko Kokubu,† Yuka Inoue,† Takashi Tsuruo,‡ and Tazuko Tashiro‡

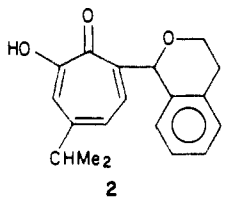
Faculty of Pharmaceutical Sciences, Okayama University, Tshushima-naka 1-1-1, Okayama 700, and Cancer Chemotherapy Center, Kami-Ikebukuro 1-37-1, Toshima-Ku, Tokyo 170, Japan. *Received August 27, 1984*

Structural requirement for antitumor activity of tropolone derivatives **2–4** was explored. Isochroman derivatives (**6–17**, **20**, and **23**) and α,α-disubstituted compounds **26–30** were synthesized and their antitumor activities were tested. These nontroponoid 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**.

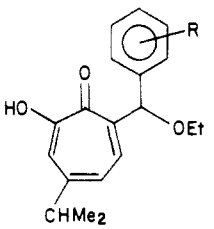
In previous papers,[1,2] we have reported the syntheses of new tropolone derivatives and their antitumor activities. Typical antitumor compounds are 3-(isochroman-1-yl)-6-isopropyltropolone (**2**), 3-(α-ethoxybenzyl)-6-isopropyltropolones (**3**), and α,α-bis(2-hydroxy-6-isopropyltropon-3-yl)toluenes (**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[3] moiety, which is a constituent of the plants of *Chamaecyparis* species.



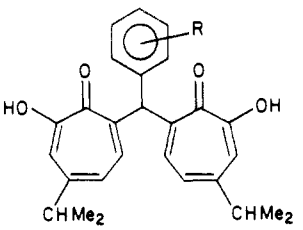
1



2



3a, R = H
b, R = OMe



4a, R = H
b, R = OMe

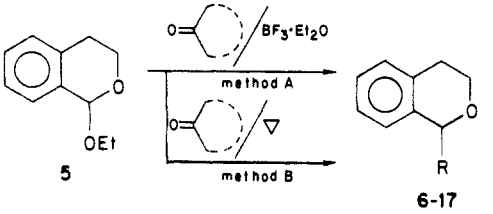
As the hinokitiol moiety is, obviously, essential to the antitumor activity, the aim of our initial effort was to develop some other effective groups that can substitute for tropolone ring in the antitumor action. Isochroman derivatives **6–17**, **20**, and **23** containing various carbonyl components or heterocyclic rings and α,α-disubstituted toluene analogues **26–30** were synthesized and their antitumor activities were tested. Consequently, these nontroponoid 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).[4–6]

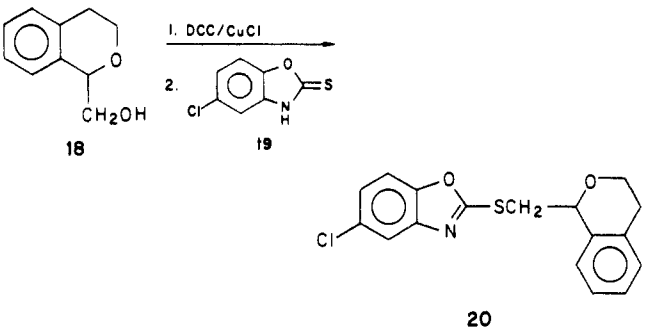
Benzoxazole derivatives **17**, **20**, and **23** were prepared by application of our finding[7] obtained from the study on the alkylation of benzoxazole derivatives. A mixture of **5** and 5-chlorobenzoxazoline-2-thione[8] (**19**) was heated to give 3-(isochroman-1-yl)benzoxazoline-2-thione (**17**) (me-

Scheme I



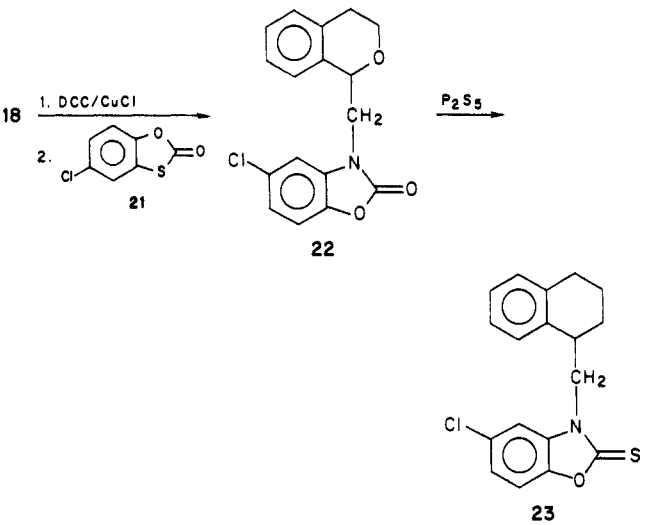
5 **6-17**

Scheme II. Method C



18 **19** **20**

Scheme III. Method D



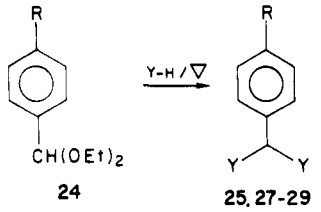
21 **22** **23**

† Okayama University.
‡ Cancer Chemotherapy Center.

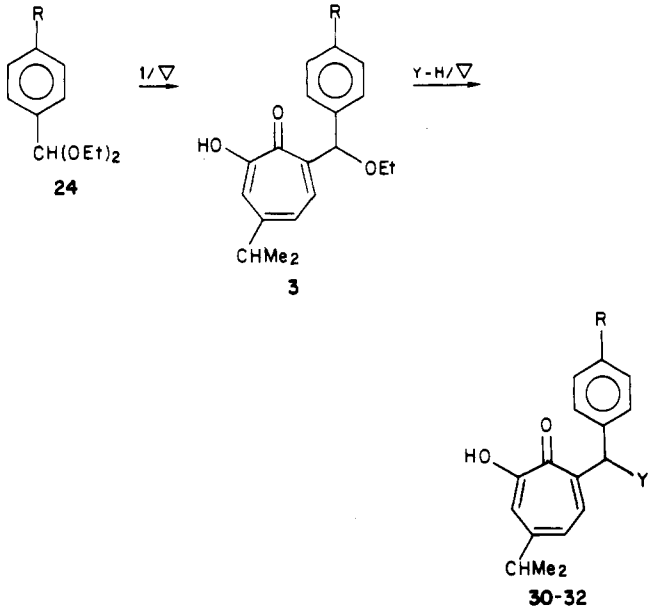
(1) Part 1: M. Yamato, K. Hashigaki, N, Kokubu, T. Tsuruo, and T. Tashiro, *J. Med. Chem.*, **27**, 1749 (1984).
(2) M. Yamato, K. Hashigaki, N, Kokubu, and Y. Nakato, *J. Chem. Soc., Perkin Trans. 1*, 1301 (1984).
(3) T. Nozoe, *Bull. Chem. Soc. Jpn.*, 11, 275 (1936).
(4) M. Yamato, T. Ishikawa, and T. Kobayashi, *Chem. Pharm. Bull.*, **28**, 2967 (1980).

0022-2623/85/1828-1026$01.50/0

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[9] (**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 α,α-bis[2-hydroxy-1-(hydroxymethyl)-4-oxo-4*H*-pyran-2-yl]-4-methoxytoluene (**29**) in a 75% yield.

3-(α-Ethoxy-4-methoxybenzyl)-6-isopropyltropolone (**3a**),[1] prepared from benzaldehyde diethyl acetal (**24a**) and hinokitiol (**1**), was heated with acetamide to give α-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-[α-(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[10] (in vitro) and antitumor activity against leukemia P388 in mice (in vivo).

As previously reported,[1] 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 isochromanylphenols **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,[11] or 2-thiobenzoxazole,[12] 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 monotropolone analogues **3**, almost comparable to that of colchicine in the in vivo system.[1] 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 nontroponoid 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 acetylamino or benzoylamino group, which is necessary for antitumor activity of colchicines,[13] gave α-acetylamino (**30**) and α-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 α-(2-hydroxy-6-isopropyltropon-3-yl)-α-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**.

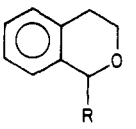
In summary, several new active compounds were prepared; however, analogues that are more active than binary tropolone derivatives **4** could not be developed.

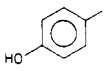
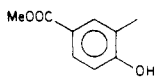
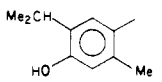
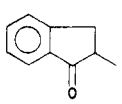
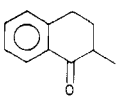
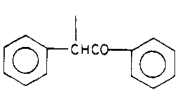
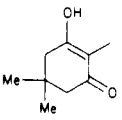
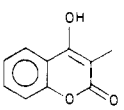
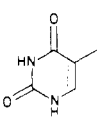
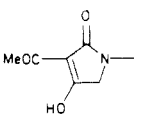
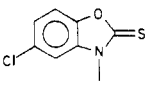
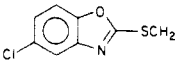
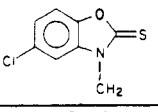
Experimental Section

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

(5) T. Ishikawa and M. Yamato, *Chem. Pharm. Bull.*, **30**, 1594 (1982).
(6) M. Yamato, T. Ishikawa, and S. Yamada, *Chem. Pharm. Bull.*, **30**, 843 (1982).
(7) M. Yamato, Y. Takeuchi, K. Hashigaki, K. Hattori, E. Muroga, and T. Hirota, *Chem. Pharm. Bull.*, **31**, 1733 (1983).
(8) A. Korczynski and S. Obavski, *Bull. Soc. Chim.*, **33**, 1823 (1923).
(9) T. Nagao, M. Ito, and K. Matsumura, *J. Am. Chem. Soc.*, **75**, 2770 (1953).
(10) T. Tsuruo, H. Iida, S. Tsukagoshi, and Y. Sakurai, *Cancer Res.*, **39**, 1063 (1979).
(11) F. A. Miller, W. A. Rightsel, B. J. Soloan, J. Ehrlich, J. C. French, and Q. R. Barte, *Nature (London)*, **200**, 1338 (1963).
(12) Y. Watanabe, Ed., "Gan To Kagakuryoho", Gan To Kagakuryohosha Press: Tokyo, 1982; Vol. 9, p 42.
(13) M. Rösner, H-G. Capraro, A. E. Jacobson, L. Atwell, A. Brossi, M. A. Iorio, T. H. Williams, R. H. Sik, and C-F. Chignell, *J. Med. Chem.*, **24**, 257 (1981); F. R. Quinn and J. A. Beisler, *ibid.*, 24, 251 (1981).

Table I. Antitumor Activity of Isochromanyl Derivatives



compd	R	ref or[a] method	crystn solvent	mp, °C	yield, %	formula	inhibn of KB cell[b] growth, ID_{50} μg/mL	nM/mL
6		4	benzene	113–115	22	$C_{15}H_{14}O_2$	27	119
7		4	MeOH	150–151	21	$C_{15}H_{14}O_2$	20.5	90
8		4	CH_2Cl_2	142–144	41	$C_{17}H_{16}O_4$	>100	352
9		A	oil		66	$C_{19}H_{22}O_2$	16.0	57
10		A	Et_2O	134–136	53	$C_{18}H_{16}O_2$	38.0	144
11		5	Et_2O	123–124	59	$C_{19}H_{18}O_2$	42.0	151
12		A	Et_2O	138–141	50	$C_{23}H_{20}O_2$	>100	305
13		5	Et_2O	120–121	80	$C_{17}H_{20}O_3$	>100	368
14		A	MeOH	167–171	71	$C_{18}H_{14}O_4$	100	340
15		6	THF	303–304	61	$C_{13}H_{12}N_2O_3$	>100	410
16		B	AcOEt–PE[c]	77–79	90	$C_{15}H_{15}NO_4$	>100	366
17		B	cyclohexane	140–141	64	$C_{16}H_{12}ClNO_2S$	85	268
20		C	AcOEt–PE	95–96	*d*	$C_{17}H_{14}ClNO_2S$	6.1	18
23		D	benzene	151–152	*d*	$C_{17}H_{14}ClNO_3$	8.9	28

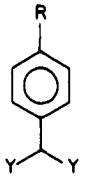
[a] The Arabic numerals show reference. [b] The compounds listed are inactive in the survival test of P388 mice at 400 mg/kg. [c] PE = petroleum ether. [d] See Experimental Section.

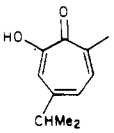
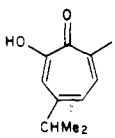
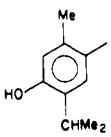
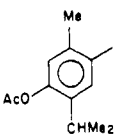
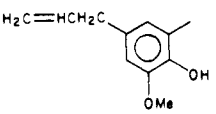
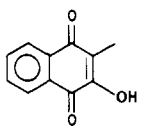
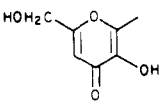
theoretical values. Column chromatographic separation were performed by flash technique.

Method A. 2-Isochroman-1-yl-1,2-dihydroinden-1-one (10). BF_3·Et_2O (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_2O (100 mL), and washed with H_2O. After the solvent was removed, the residue was crystallized from Et_2O to give **10** (10.6 g, 53%): mp 134–136 °C; IR (Nujol) 1710 cm^{-1}; NMR ($CDCl_3$) δ 2.3–3.6 (m, 5 H, 2-H, 3-H_2, 4′-H_2), 3.6–4.3 (m, 2 H, 3′-H_2), 5.58 (d, 1 H, 1′-H, J = 2 Hz), 7.0–8.0 (m, 8 H, aromatic H); MS, m/z 264 (M^+). Anal. ($C_{18}H_{16}O_2$) C, H.

Method B. 5-Chloro-3-isochroman-1-ylbenzoxazoline-2-thione (17). A solution of **5** (4 g, 23 mmol) and 5-chlorobenzoxazoline-2-thione[8] (**19**; 3.1 g, 17 mmol) in dry xylene (200 mL) was refluxed for 5 h, washed with H_2O, and concentrated. The residue was crystallized from cyclohexane to give **17** (3.4 g, 64%): mp 140–141 °C; UV ($CHCl_3$) λ_{max} 314 nm (ε 30199), 268 (10232), 262 (10715); NMR ($CDCl_3$) δ 2.6–3.6 (m, 2 H, 4′-H_2), 3.9–4.5 (m, 2 H, 3′-H_2), 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_{50} μg/mL	inhibn of KB cell growth, ID_{50} nM/mL	antitumor act. P388 in mice, ip[a] doses, mg/kg	antitumor act. P388 in mice, ip[a] T/C, %
4a[b]	H		0.3	0.7	5 2.5 1.3	188 150 132
4b[b]	OMe		0.5	1.1	5 2.5 0.6	173 134 127
25	H		13	34	400 200 100	102 100 87
26	COMe		55	107	400 200 100	88 89 91
27	H		55	132	400 200 100	83 100 91
28	H		76	163	50 25 12	97 95 103
29	OMe		100	249	200 100 50	102 98 102

[a] The doses listed was given once a day for 1 and 5 days. [b] 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[14] (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 ($CDCl_3$) δ 2.6–3.0 (m, 2 H, 4′-H_2), 3.01 (s, 1 H, OH), 3.5–4.0 (m, 4 H, 3′-H_2, CH_2OH), 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.

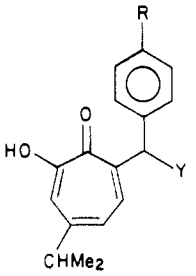
Method C. 5-Chloro-2-[(isochroman-1-ylmethyl)thio]benzoxazole (20). A mixture of **18** (1.8 g, 11 mmol), *N,N′*-dicyclohexylcarbodiimide (DCC; 2.5 g, 12 mmol), CuCl (catalytic amount), and tetrahydrofuran (THF; 100 mL) was stirred at room temperature for 12 h and 5-chlorobenzoxazoline-2-thione[8] (**19**; 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) δ 2.6–3.1 (m, 2 H, 4′-H_2), 3.3–4.3 (m, 4 H, 3′-H_2, SCH_2), 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)benzoxazoline-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[9] (**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 ($CDCl_3$) δ 2.7–3.0 (2 H, m, 4′-H_2), 3.5–4.5 (m, 4 H, 3′-H_2, NCH_2), 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.

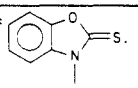
A suspension of **22** (2.8 g, 9 mmol) and P_2S_5 (5.5 g, 25 mmol) in xylene (100 mL) was refluxed for 9 h and the hot reaction mixture was filtered. To the filtrate was added Et_2O and the Et_2O solution was washed with concentrated NH_3 solution. After the solvent was removed, the residue was crystallized 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.

(14) N. N. Vorozhtsov, Jr., and A. T. Petushkova, *Zh. Obshch. Khim.*, **27**, 2282 (1957).

Table III. Antitumor Activity of Monotropolone Derivatives



compd	R	Y	inhibn of KB cell growth, ID_{50} μg/mL	inhibn of KB cell growth, ID_{50} nM/mL	antitumor act. P388 in mice, ip[a] doses, mg/kg	antitumor act. P388 in mice, ip[a] T/C, %
3a[b]	H	OEt	0.3	1.0	400	0
					200	109
					100	102
					50	97
					25	96
					12.5	96
3b[b]	OMe	OEt	0.5	1.5	400	140
					200	128
					100	140
					50	119
					25	109
					12.5	96
30	OMe	NHCOMe	6.0	18.0	400	108
					200	92
					100	92
31	OMe	$NHCOC_6H_5$	1.7	4.2	400	131
					200	110
					100	104
32	OMe	Boz[c]	3.7	8.5	400	133
					200	105
					100	113
33	H	SC_6H_5	0.5	1.4	400	113
					200	135
					100	108

[a] The doses listed were given once a day for 1 and 5 days. [b] See ref 1. [c] Boz = 

α,α-Bis(4-hydroxy-5-isopropyl-2-methoxyphenyl)toluene (25). BF_3·Et_2O (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_2O, and recrystallized from benzene to give **25** (14.5 g, 75%): mp 169–172 °C; NMR ($CDCl_3$) 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, C*H*Me_2 × 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^+). Anal. ($C_{27}H_{32}O_2$) C, H.

α,α-Bis(4-acetoxy-5-propyl-2-methylphenyl)-4-acetyltoluene (26). To a solution of $AlCl_3$ (1.1 g, 8.2 mmol) in dry CS_2 (20 mL) was added a solution of **27** (3 g, 7.7 mmol) and acetyl chloride (0.65 g, 8.3 mmol) in CS_2 (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_2O. 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^{-1}; NMR ($CDCl_3$) δ 1.00 (d, 12 H, CHMe_2 × 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^+). Anal. ($C_{33}H_{38}O_5$) C, H.

Method E. α,α-Bis[3-hydroxy-6-(hydroxymethyl)-4-oxo-4*H*-pyran-2-yl]-4-methoxytoluene (29). A mixture of *p*-anisaldehyde 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^{-1}; NMR (Me_2SO-d_6) δ 3.72 (s, 3 H, OMe), 4.31 (s, 4 H, CH_2OH × 2), 5.25 (br s, 2 H, CH_2OH × 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. ($C_{20}H_{18}O_9$) 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_2O); yield 23%; NMR ($CDCl_3$) δ 3.27 (d, 4 H, C*H*$_2$CH=CH_2 × 2, J = 6 Hz), 3.76 (s, 6 H, OMe × 2), 4.7–5.3 (m, 4 H, CH_2CH=C*H*$_2$ × 2), 5.69 (s, 2 H, OH × 2), 5.7–6.2 (m, 2 H, CH_2C*H*=CH_2 × 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_6H_5); MS, m/z 416 (M^+).

2,2′-Benzylidenebis(3-hydroxy-1,4-naphthoquinone) (28): mp 232–234 °C (from EtOH–dioxane); yield 83%; NMR (Me_2SO-d_6) 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^+). Anal. ($C_{28}H_{18}O_7$) C, H.

Method F. 3-[α-(Acetylamino)-4-methoxybenzyl]-6-isopropyltropolone (30). A mixture of 3-(α-ethoxy-4-methoxybenzyl)-6-isopropyltropolone[2] (**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^{-1}; NMR (pyridine-d_5) δ 1.15 (d, 6 H, CHMe_2, J = Hz), 2.15 (s, 3 H, COMe), 2.5–3.2 (m, 1 H, C*H*Me_2), 3.64 (s, 3 H, OMe), 6.7–8.2 (m,

9 H, α-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-[α-(Benzoylamino)-4-methoxybenzyl]-6-isopropyltropolone (31): mp 172–173 °C (from CH_2Cl_2–MeOH); yield 53%; NMR ($CDCl_3$) δ 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, α-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. ($C_{25}H_{25}NO_4$) C, H, N.

3-[α-(2-Hydroxy-6-isopropyltropon-3-yl)-4-methoxybenzyl]benzoxazoline-2-thione (32): mp 163–166 °C (from CH_2Cl_2–MeOH); yield 41%; IR (Nujol) 3150; NMR ($CDCl_3$) δ 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-[α-(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 ($CDCl_3$) δ 1.28 (d, 6 H, $CHMe_2$, J = 7 Hz), 2.6–3.2 (m, 1 H, $CHMe_2$), 6.58 (s, 1 H, α-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.[1]

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.

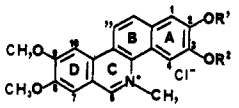
Synthesis and Antitumor Activity of Structural Analogues of the Anticancer Benzophenanthridine Alkaloid Fagaronine Chloride

Mark Cushman* and Prem Mohan

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907. *Received November 29, 1984*

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 nitidum*[1,2] and *Fagara zanthoxyloides*,[3] respectively. The structure elucidation of nitidine (1) involved its chemical conversion to known compounds[2] as well as the synthesis of dihydronitidine,[4] while that of fagaronine (2) was proposed after NMR analysis of its *N*-demethyl derivative[5] and confirmed by total synthesis.[6] Nitidine (1) has also been synthesized by several methods.[4,7]



1, $R^1 + R^2 = CH_2$
2, $R^1 = H$; $R^2 = CH_3$

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

Table I. Evaluation of the Indenoisoquinoline Analogue 3 of Nitidine (1) for Anticancer Activity in the M5076 Sarcoma System[a]

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

[a] For the general screening procedure and data interpretation, see ref 19.

(1) Arthur, H. R.; Hui, W. H.; Ng, Y. L. *Chem. Ind. (London)* **1958**, 1514.
(2) Arthur, H. R.; Hui, W. H.; Ng, Y. L. *J. Chem. Soc.* **1959**, 1840.
(3) Messmer, W. M.; Tin-Wa, M.; Fong, H. H. S.; Bevelle, C.; Farnsworth, N. R.; Abraham, D. J.; Trojanek, J. *J. Pharm. Sci.* **1972**, *61*, 1858.
(4) (a) Arthur, H. R.; Ng, Y. L. *J. Chem. Soc.* **1959**, 4010. (b) Gopinath, K. W.; Govindachari, T. R.; Partasarathy, P. G.; Viswanathan, N. *J. Chem. Soc.* **1959**, 4012. (c) Ninomiya, I.; Naito, T.; Ishii, H.; Ishida, T.; Ueda, M.; Harada, K. *J. Chem. Soc., Perkin Trans. 1* **1975**, 762.
(5) Tin-Wa, M.; Bell, C. L.; Bevelle, C.; Fong, H. H. S.; Farnsworth, N. R. *J. Pharm. Sci.* **1974**, *63*, 1476.
(6) (a) Gillespie, J. P.; Amoros, L. G.; Stermitz, F. R. *J. Org. Chem.* **1974**, *39*, 3239. (b) Ishii, H.; Chen, I.-S.; Ishikawa, T. *Chem. Pharm. Bull.* **1983**, *31*, 2963.
(7) (a) Zee-Cheng, K.-Y.; Cheng, C. C. *J. Heterocycl. Chem.* **1973**, *10*, 85. (b) Kametani, T.; Kigasawa, K.; Hiiragi, M.; Kusana, O. *Ibid.* **1973**, *10*, 31. (c) Kessar, S. V.; Singh, G.; Salakrishnan, P. *Tetrahedron Lett.* **1974**, 2269. (d) Begley, W.; Grimshaw, J. *J. Chem. Soc., Perkin Trans. 1* **1977**, 2324. (e) Cushman, M.; Cheng, L. *J. Org. Chem.* **1978**, *43*, 286. (f) Dyke, S. F.; Sainsbury, M.; Moon, B. J. *Tetrahedron* **1968**, *24*, 1467.

0022-2623/85/1828-1031$01.50/0