73090-70-7; **9**, 78025-72-6; **10**, 77113-54-3; 11, 20344-69-8; **12**, 100515-03-5; **13**, 107698-02-2; **14**, 107698-00-0; **15**, 100515-04-6; **16**, 71525-05-8; **17**, 69945-54-6; **18**, 73356-41-9; **19**, 13932-40-6; **20**, 5355-16-8; **21**, 83166-76-1; **22**, 107698-03-3; **23**, 73356-40-8; **24**, 107697-99-4; **25**, 83158-06-9; **26**, 107698-04-4; **27**, 78233-99-5; **28**, 50823-96-6; **29**, 98612-09-0; **30**, 98612-08-9; **31**, 80407-58-5; **32**, 77113-56-5; **33**, 77113-58-7; **34**, 77113-57-6; **35**, 77113-55-4; **36**, 80416-29-1; **37**, 59481-28-6; **38**, 69945-57-9; **39**, 69945-56-8; **40**, 69945-58-0; **41**, 69945-59-1; **42**, 50823-94-4; **43**, 77113-61-2; **44**, 30077-60-2; **45**, 77113-63-4; **46**, 69945-60-4; **47**, 77113-62-3; **48**, 80407-62-1; **49**, 77113-60-1; **50**, 69945-50-2; **51**, 69945-53-5; **52**, 107698-01-1; **53**, 30077-67-9; **54**, 80407-59-6; **55**, 69945-52-4; **56**, 20285-70-5; **57**, 836-06-6; **58**, 69945-51-3; **59**, 46726-70-9; **60**, 18588-43-7; **61**, 69945-55-7; **62**, 49561-94-6; **63**, 77113-59-8; **64**, 49873-11-2; **65**, 80407-61-0; **66**, 80407-60-9; **67**, 93317-64-7; **68**, 7319-45-1; DHFR, 9002-03-3.

Absolute Structure–Cytotoxic Activity Relationships of Steganacin Congeners and Analogues[1]

Kiyoshi Tomioka,[†] Tsuneo Ishiguro,[†] Hidemichi Mizuguchi,[†] Nobuyasu Komeshima,[†] Kenji Koga,*[,†] Shigeru Tsukagoshi,[‡] Takashi Tsuruo,[‡] Tazuko Tashiro,[‡] Seiichi Tanida,[§] and Toyokazu Kishi[§]

Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan, Cancer Chemotherapy Center, Cancer Institute, Kamiikebukuro, Toshima-ku, Tokyo 170, Japan, and Central Research Divisions, Takeda Chemical Industries, Ltd., 17-85 Jusohonmachi 2-chome, Yodogawaku, Osaka 532, Japan. *Received January 19, 1990*

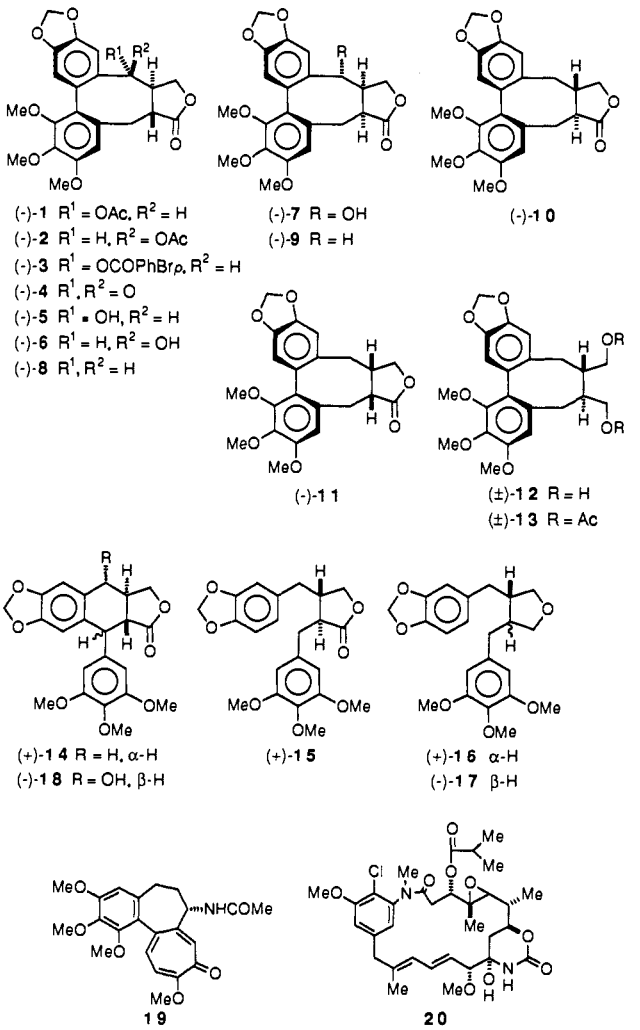
The cytotoxic activities of optically pure and racemic steganacin congeners and analogues against KB cells in culture and the inhibitor activity of cilia regeneration in *Tetrahymena* were studied with regard to absolute and relative configurations. The stereochemical requirements of dibenzocyclooctadiene lignan lactones for activity were clarified.

Steganacin [(−)-1], a naturally occurring dibenzocyclooctadiene lignan lactone, has been reported to show significant antitumor activity in vivo against P388 leukemia in mice and in vitro against cells derived from a human carcinoma of the nasopharnyx (KB).[2] It has been suggested that steganacin, like other spindle poisons, such as the ansamitocins (maytansine),[3] colchicine,[4] and podophyllotoxin,[5] exerts its antimitotic activity through an effect on spindle microtubules.[6]

Absolute structure–activity relationships of chiral compounds have been focus in recent medicinal chemistry. We have been involved in recent years with the asymmetric total synthesis of lignans and have found that the absolute configuration of natural (−)-steganacin had to be corrected and drawn as (−)-**1**, contrary to the antipodal structure proposed by Kupchan.[7] Since all of the possible optically pure enantiomers of steganacin congeners and analogues can be prepared by using the asymmetric synthesis we developed, the study of structure–activity relationships in enantiomers allow us to obtain greater insight into the structural requirements for antitumor activity. We report here that the correct absolute configuration around the pivotal bond and that the orientation of the lactone carbonyl are critical for expression of the antitumor activity of dibenzocyclooctadiene lignan lactones.

The dibenzocyclooctadiene compounds (±)-, (−)-, and (+)-steganacin (**1**),[7] episteganacin (**2**),[7] stegane (**8**),[7,8] picrostegane (**9**),[8] isostegane (**10**),[8] isopicrostegane (**11**),[8] (±)- and (−)-steganol (**5**),[7] (−)-[(*p*-bromobenzoyl)oxy]stegane (**3**),[7] (−)-steganone (**4**),[7] (−)-episteganol (**6**),[7] and (−)-picrosteganol (**7**)[9] (Chart I) were prepared as described previously. Isostegane derivatives (±)-**12**, **13**,[8] (±)- and (−)-isodeoxypodophyllotoxin (**14**),[10] (±)-, (−)-, and (+)-deoxypodorhizone (**15**),[7] (−)- and (+)-burseran (**16**), and (−)-**17**,[11] were also prepared as described. Steganacin derivatives (−)-**21**–**25** (Chart II) were prepared from (−)-**5**,[7] respectively, as described in the Experimental Section.

Chart I

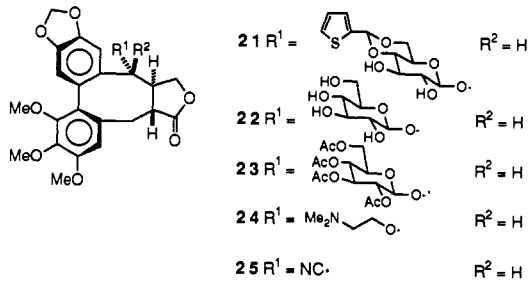


Natural (−)-podophyllotoxin (**18**) and (−)-colchicine (**19**) were purchased from Aldrich Chemical Co. and purified

[†] University of Tokyo.
[‡] Cancer Institute.
[§] Takeda Chemical Industries Ltd.

0022-2623/91/1834-0054$02.50/0

Chart II



by silica gel column chromatography.

Cytotoxicity

The initial in vitro screening of this series of 37 compounds was carried out with KB cells derived from a human epidermoid carcinoma of the mouth. In all cases, an ED_{50} of 4 μg/mL upon testing for confirmation of the initial activity was sufficient to consider the series member active against KB cells. The results of testing are listed in Tables I–III. The activities of the standards (−)-podophyllotoxin (**18**) and (−)-colchicine (**19**) are also listed.

As shown in Table I for absolute structure–activity comparison purposes, among (±)-, (−)-, and (+)-series of **1**, **2**, and **8**–**11**, the compounds showing higher activity are the (−)-series of compounds with the same *R* absolute configuration around the pivotal biphenyl bond. The configuration of the acetoxy group is also critical because α-substitution (**1**) exerts a higher activity than β-substitution (**2**). It is noteworthy that (−)-**11** shows an activity parallel to that of (−)-**1** and higher than that of (−)-**8**, the parent skeleton of (−)-**1**.

The substituents of (−)-stegane (**8**) exert profound effects on activity. As shown in Table II for various substitutions on the benzylic position of (−)-**8** and (−)-**9**, relatively large substituents are responsible for the decreased activity. For example, glucosides **21**–**23** show quite marginal activity.

Table I. Effects of (±)-, (−)-, and (+)-Steganacin Congeners and Analogues on Inhibition of KB Cell Growth (ED_{50}) and Cilia Regeneration in *Tetrahymena* (MIC)[a]

compound	ED_{50}, μg/mL	MIC, μg/mL
(±)-steganacin	<0.3	>20
(−)-steganacin (**1**)	<0.3	>20 (100 mc)
(+)-steganacin	1.75	>20
(±)-episteganacin	15.7	>20
(−)-episteganacin (**2**)	16.6	>20
(+)-episteganacin	61.0	>20
(±)-stegane	0.94	>20
(−)-stegane (**8**)	0.49	>20
(+)-stegane	86.0	>20
(±)-picrostegane	28.0	>20
(−)-picrostegane (**9**)	8.8	>20
(+)-picrostegane	32.0	>20
(±)-isostegane	1.33	>20
(−)-isostegane (**10**)	0.47	>20
(+)-isostegane	94.0	>20
(±)-isopicrostegane	<0.3	20 (40)
(−)-isopicrostegane (**11**)	<0.3	10–20 (>40)
(+)-isopicrostegane	4.1	>20 (40)
(−)-podophyllotoxin (**18**)	<0.3	>20
(−)-colchicine (**19**)	<0.3	>20 (>40)
ansamitocin P-3 (**20**)	n	≤0.5 (2)

[a] Numbers in parentheses are MIC for inhibition of growth of *Tetrahymena pyriformis* W; mc = morphorogical change (rounding); n = not tested.

Table II. Effects of (−)-Steganacin Analogues on Inhibition of KB Cell Growth

compd	R^1	R^2	ED_{50}, μg/mL
1	OAc	H	<0.3
2	H	OAc	16.6
3	OCOPhBr-*p*	H	3.9
4	O	O	6.2
5	OH	H	0.53
6	H	OH	5.0
7[a]	OH	H	16.0
8	H	H	0.49
9[a]	H	H	8.8
21	sugar	H	>100
22	sugar	H	>100
23	sugar	H	56
24	$O(CH_2)_2NMe_2$	H	1.50
25	CN	H	1.52

[a] Picrostegane skeleton.

Table III. Effects of Steganacin and Podophyllotoxin Congeners and Analogues of Inhibition of KB Cell Growth (ED_{50}) and Cilia Regeneration in *Tetrahymena* (MIC)[a]

compound	ED_{50}, μg/mL	MIC, μg/mL
(±)-isostegane (**10**)	1.13	>20
(±)-isostegane diol (**12**)	75.0	n
(±)-isostegane diacetate (**13**)	15.0	n
(−)-isopicrostegane (**11**)	<0.3	10–20 (>40)
(±)-isodeoxypodophyllotoxin (**14**)	<0.3	>20
(−)-isodeoxypodophyllotoxin	1.75	>20
(±)-deoxypodorhizon	16.8	>20
(−)-deoxypodorhizon (**15**)	n	>20
(+)-deoxypodorhizon	5.2	20 (≤20)
(−)-*trans*-burseran	n	≥20 (>40)
(+)-*trans*-burseran (**16**)	n	20 (40)
(−)-*cis*-burseran (**17**)	n	≥20 (40)

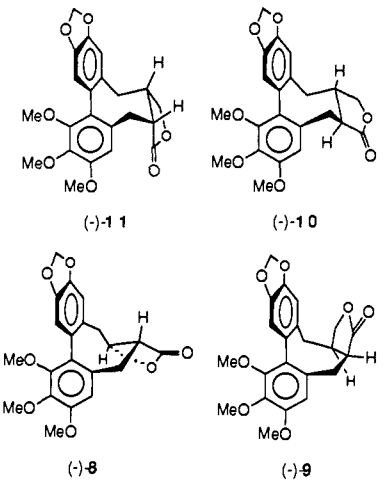
[a] Numbers in parentheses are MIC for inhibition of growth of *T. pyriformis* W; n = not tested.

The lactone ring is probably essential for exerting activity. As shown in Table III, diol **12** obtained by lithium aluminum hydride reduction of (±)-**10** was inactive and its diacetate (**13**) was also inactive.

(−)-Isodeoxypodophyllotoxin (**14**) was confirmed to show weaker activity than (+)-**14**, since (±)-**14** showed potent activity (Table III).

(1) Stereoselective Reactions. Part XVII. For part XVI, see: Tomioka, K.; Tanaka, M.; Koga, K. *Chem. Pharm. Bull.* **1989**, *37*, 1201.
(2) Isolation: Kupchan. S. M.; Britton, R. W.; Ziegler, M. F.; Gilmore, C. J.; Restivo, R. J.; Bryan, R. F. *J. Am. Chem. Soc.* **1973**, *95*, 1335. Recent synthesis: Meyers, A. I.; Flisak, J. R.; Aitken, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 5446 and references cited therein.
(3) Tanida, S.; Higashide, E.; Yoneda, M. *Antimicrob. Agents Chemother.* **1979**, *16*, 101; Higashide, E.; Asai, M.; Ootsu, K.; Tanida, S.; Kozai, Y.; Hasegawa, T.; Kishi, T.; Sugino, Y.; Yoneda, M. *Nature* **1977**, *270*, 721. Kupchan, S. M.; Komoda, Y.; Court, W. A.; Thomas, G. J.; Smith, R. M.; Karin, A.; Gilmore, C. J.; Haltiwanger, R. C.; Bryan, R. F. *J. Am. Chem. Soc.* **1972**, *94*, 1354.
(4) Wilson, L. *Biochemistry*, **1970**, *9*, 4999.
(5) Jardine, I. *Anticancer Agents Based on Natural Product Models*; Cassady, J. M., Douros, J. D., Eds.; Academic Press: New York 1980; p 319.
(6) Zavala, F.; Guenard, D.; Robin, J.-P.; Brown, E. *J. Med. Chem.* **1980**, *23*, 546, Schiff, P. B.; Kende, A. S.; Horwitz, S. B. *Biochem. Biophys. Res. Commun.* **1978**, *85*, 737. Wang, R. W. J.; Rebbun, L. I.; Kupchan, S. M. *Cancer Res.* **1977**, *37*, 3071.
(7) Tomioka, K.; Ishiguro, T.; Iitaka, Y.; Koga, K. *Tetrahedron* **1984**, *40*, 1303.
(8) Tomioka, K.; Mizuguchi, H.; Ishiguro, T.; Koga, K. *Chem. Pharm. Bull.* **1985**, *33*, 121.
(9) Tomioka, K.; Ishiguro, T.; Iitaka, Y.; Koga, K. *Chem. Pharm. Bull.* **1986**, *34*, 1501.
(10) Tomioka, K.; Koga, K. *Tetrahedron Lett.* **1979**, 3315.
(11) Tomioka, K.; Ishiguro, T.; Koga, K. *Chem. Pharm. Bull.* **1985**, *33*, 4333.

Chart III



Inhibition of Cilia Regeneration in *Tetrahymena*

The next test was carried out by examining cilia regeneration in *Tetrahymena*. Cilia regneration in *Tetrahymena* is a useful system for determining the antitubulinic properties of spindle poisons such as ansamitocins (**20**).[12] The results are summarized in Tables I and III. The activity of ansamitocin P-3 (**20**) is also shown as a standard. Among the 32 compounds tested, (-)-**11**, (+)-**15**, (-)- and (+)-**16**, and (-)-**17** showed significant effects on regeneration. (-)-Isopicrostegane [(-)-**11**] showed the highest inhibitory effect on regeneration, suggesting that the cytotoxic activity of (-)-**11** is based on an effect on spindle microtubules. It is also interesting to note that (+)-**15**, (-)- and (+)-**16**, and (-)-**17** showed inhibitory activity. The reported antitumor effect of **16** is presently explained through an effect of spindle microtubules.[13]

Discussion

The steganine lignans have four structural features: (1) chirality around the pivotal biphenyl bond, (2) conformation of the eight-membered carbocycle, (3) chirality around the lactone, and (4) chirality at the benzylic position γ to the lactone carbonyl.

Among these features, the chirality around the pivotal bond is the most essential for activity. As shown in Table I, the more active enantiomer has an *R* configuration around the pivotal bond. This absolute spatial arrangement of the two aromatic rings is consistent with those of natural podophyllotoxin (**18**) and colchicine (**19**).[6] However, (+)-steganacin [(+)-**1**] of *S* configuration around the pivotal bond still maintains sufficient activity and the benzylic substituent OAc of (+)-**1** seems to be responsible for its high activity since the parent skeleton (+)-**8** exhibits only marginal activity. This is in sharp contrast to the quite low activity of all isomers of **2** and the lower activity of (-)-**7** than (-)-**9**.

It is interesting to note that substituents at the benzylic position γ to the lactone carbonyl of **1** show a profound effect on activity (Table II) and that the large substituents cause decreased activity. This feature is quite different from the podophyllotoxin series in which an analogous glucose substituent provides significant activity.[14]

Two of the features, conformation of the eight-membered carbocycle and chirality around the γ-lactone, are related to each other. The rigid conformations of dibenzocyclooctadienes (-)-**8**–**11** are presented in Chart III.[15] These conformations were obtained by inspecting molecular models and are supported by X-ray crystallographic structure determination of derivatives of these compounds.[2,7,9] Two of the four compounds, **8** and **9**, have a boat–boat conformation, and **10** and **11** have a boat–chair conformation. Since only **9** exhibits relatively low activity, the conformation of the eight-membered ring is not directly responsible for the activity. However, the major difference between **9** and **8**, **10**, and **11** is the spatial arrangement of the aromatic rings and the lactone carbonyl group. The carbonyl oxygen of **8**, **10**, and **11** is placed far away from the methylenedioxyphenyl ring and the C–O double bond of **9** has a parallel orientation to the (methylenedioxy)phenyl ring. The reduced activity of diol **12**, obtained from (±)-**10**, also suggests that the carbonyl oxygen is responsible for the activity.

These analyses suggest that, in exerting an effect on spindle microtubules, the compounds have the following stereochemical requirements: the absolute spatial arrangement of the two aromatic rings is an *R* configuration and the lactone carbonyl oxygen is placed far away from one of the aromatic rings, possibly similar to the orientation shown in (-)-**11** Chart III. Two aromatic rings would most likely fit in to the hydrophobic pocket in a restricted stereochemical requirement and the lactone carbonyl oxygen plays the role of an acceptor of the hydrogen bond.

According to these guidelines, we are searching for artificial compounds with more ideal structure and activity.[16]

Experimental Section[17]

(-)-4α-[[4,6-*O*-(2-Thienylmethylene)-β-D-glucopyranosyl]oxy]stegane [(-)-21]. A mixture of (-)-**22** (111 mg, 0.193 mmol), thiophene-2-carboxaldehyde (1 mL) and $ZnCl_2$ (60 mg, 0.44 mmol) was stirred at room temperature for 2 h and diluted with $CHCl_3$. The mixture was washed with water and saturated NaCl and dried over Na_2SO_4. Concentration and dilution with hexane (100 mL) and filtration afforded a white solid. The solid was dissolved in $CHCl_3$–MeOH and again diluted with hexane to provide a white solid. Filtration provided (-)-**21** (96 mg) as a white powder in 74% yield: $[\alpha]^{22}_D = -107°$ (*c* 0.178, $CHCl_3$–MeOH (9:1)); IR (KBr) 1460, 1763, 1580, 1480 cm^{-1}; NMR (DMSO-d_6) δ 2.1–4.9 (12 H, m), 5.1–5.3 (2 H, m), 3.25 (2 H, s, OH), 3.55, 3.74, and 3.77 (each 3 H, s, OCH_3), 5.74 (1 H, s, C*H*), 6.01 and 6.02 (each 1 H, d, J = 1 Hz, OCH_2O), 6.28, 6.35, and 6.92 (each 1 H, s, Ar*H*), 6.92, 7.07, and 7.43 (each 1 H, m, thiophene-H). Anal. ($C_{33}H_{34}O_{13}S$) C, H.

(-)-4α-(β-D-Glucopyranosyloxy)stegane [(-)-22]. A mixture of (-)-**23** (240 mg, 0.322 mmol) and $Zn(OAc)_2$ (56 mg, 0.31 mmol)

(12) Tanida, S.; Hasegawa, T.; Higashide, E. *Agric. Biol. Chem.* **1980**, *44*, 1847.
(13) Cole, J. R.; Bianchi, E.; Trumbell, E. R. *J. Pharm. Sci.* **1969**, *58*, 175.
(14) Long, B. H.; Minoccha, A. *Proc. Am. Assoc. Cancer Res.* **1983**, *24*, 1271.
(15) Ishiguro, T.; Mizuguchi, H.; Tomioka, K.; Koga, K. *Chem. Pharm. Bull.* **1985**, *33*, 609.
(16) According to these considerations, we have succeeded in the design and synthesis of artificial azasteganes and azapodophyllotoxins: Tomioka, K.; Kubota, Y.; Kawasaki, H.; Koga, K. *Tetrahedron Lett.* **1989**, *30*, 2949. Tomioka, K.; Kubota, Y.; Koga, K. *Ibid.* **1989**, *30*, 2953, *Idem. J. Chem. Soc., Commun.* **1989**, 1622.
(17) Melting points were measured with a Büchi 510 melting point apparatus and are not corrected. Optical rotations were taken with a JASCO DIP-181 polarimeter. IR spectra were taken with a JASCO infrared spectrometer Model DS-402G. ^1H NMR spectra were taken in $CDCl_3$ with a JEOL GX-400 spectrometer at 400 MHz, a JNM-PS 100 spectrometer, a JEOL-FX 100 spectrometer at 100 MHz, or with an Hitachi R-24 spectrometer at 60 MHz. Chemical shift values are expressed in ppm relative to internal tetramethylsilane. Abbreviations are as follows: s, singlet, d, doublet; t, triplet, m, multiplet. MS were taken with a JEOL-01 SG-2 mass spectrometer or a JEOL DX-300 mass spectrometer.

in EtOH (4 mL) was stirred at reflux for 14 h and diluted with $CHCl_3$-*i*-PrOH (4:1, 50 mL). The mixture was washed with water and saturated NaCl and dried over Na_2SO_4. Concentration and silica gel column chromatography ($CHCl_3$–MeOH (10:1)) afforded (-)-**22** (143 mg) as a white solid with a melting point of 219° (dec) in 77% yield: $[\alpha]^{22}_D$ = -130° (*c* 0.152, $CHCl_3$–MeOH (9:1)); IR (KBr) 3400, 1752 cm^{-1}; NMR ($CDCl_3$–CD_3OD (9:1)) δ 2.3–2.8 (3 H, m), 2.8–5.0 (8 H, m), 3.75, 3.84, and 3.87 (each 3 H, s, OCH_3), 5.96 (2 H, s, OCH_2O), 6.38, 6.46, and 6.84 (each 1 H, s, Ar*H*); MS *m/z* 576 (M^+). Anal. ($C_{28}H_{32}O_{13}$) C, H.

(-)-4α[(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)oxy]stegane [(-)-23]. A solution of (-)-steganol (**5**[8], 204 mg, 0.49 mmol), tetra-*O*-acetyl-α-D-glucose[18] (308 mg, 0.88 mmol), and BF_3–Et_2O (181 mg, 1.28 mmol) in 1,2-dichloroethane (23 mL) was stirred at -20 °C for 1 h and then quenched with pyridine (0.5 mL). After dilution with ethyl acetate (50 mL), the mixture was washed with saturated NaCl and dried over $MgSO_4$. Concentration and purification by silica gel column chromatography (benzene–ethyl acetate (3:1)) afforded (-)-**23** (309 mg) as a colorless amorphous in 85% yield: $[\alpha]^{20}_D$ = -119° (*c* 0.91, $CHCl_3$); IR (KBr) 1754 cm^{-1}; NMR ($CDCl_3$) δ 1.90 (6 H, s, Ac), 1.94 and 1.99 (each 3 H, s, Ac), 2.2–3.2 (4 H, m), 3.3–5.2 (10 H, m), 3.84, 3.87, and 3.98 (each 3 H, s, OCH_3), 5.98 (2 H, s, OCH_2O), 6.40, 6.43, and 6.70 (each 1 H, s, Ar*H*; MS *m/z* 744 (M^+). Anal. ($C_{36}H_{40}O_{17}$) C, H.

(-)-4α-[2-(*N*,*N*-Dimethylamino)ethoxy]stegane [(-)-24]. A suspension of (-)-**5**[8] (23.7 mg, 0.057 mmol), *N*,*N*-dimethyl-2-hydroxyethylamine (14.6 mg, 0.60 mmol), BF_3–Et_2O (207 mg, 1.40 mmol), and 4A molecular sieve (1 g) in 1,2-dichloroethane (8 mL) was stirred at room temperature for 3 h. After dilution with ethyl acetate (30 mL), the mixture was washed with 10% NaOH and saturated NaCl and dried over $MgSO_4$. Concentration and purification by silica gel column chromatography ($CHCl_3$–MeOH (4:1)) afforded (-)-**24** (6.3 mg) as an oil in 23% yield: $[\alpha]^{22}_D$ = -125° (*c* 0.32, $CHCl_3$); IR ($CHCl_3$) 1770 cm^{-1}; NMR ($CDCl_3$) δ 2.25 (6 H, s, $N(CH_3)_2$), 2.2–4.8 (11 H, m), 3.70, 3.82, and 3.87 (each 3 H, s, OCH_3), 5.98 (2 H, s, OCH_2O), 6.35, 6.51, 6.68 (each 1 H, s, Ar*H*); MS *m/z* 485 (M^+). Anal. ($C_{26}H_{31}NO_8$) C, H, N.

(-)-4α-Cyanostegane [(-)-25]. A solution of (-)-steganol (**5**[8]; 77.9 mg, 0.19 mmol), tetra-*O*-acetyl-α-D-glucopyranosyl bromide[16] (124 mg, 0.30 mmol), and mercuric cyanide (84 mg, 0.332 mmol) in acetonitrile (2 mL) was stirred at reflux for 3 h. After dilution with ethyl acetate (20 mL), the mixture was washed with saturated NaCl and dried over $MgSO_4$. Concentration and purification by silica gel column chromatography (hexane–ethyl acetate (3:2)) afforded (-)-**25** (35.4 mg) as a solid in 45% yield: mp 233.5–234.5 °C; $[\alpha]^{20}_D$ = -201° (*c* 0.40, $CHCl_3$). IR (KBr) 2230, 1775 cm^{-1}; NMR ($CDCl_3$) δ 1.8–2.2 (2 H, m, $ArCH_2$), 2.3–3.2 (3 H, m, C*H* × 3), 3.6–4.7 (2 H, m, CH_2O), 3.76, 3.85, and 3.88 (each 3 H, s, OCH_3), 5.99 (2 H, s, OCH_2O), 6.43, 6.60, and 6.68 (each 1 H, s, ArH). MS *m/z* 423 (M^+). Anal. ($C_{23}H_{21}NO_7$) C, H, N.

(18) Lemieux, R. U. *Methods in Carbohydrate Chemistry*; Whister, R. L., Wolfrom, M. L., Eds.; Academic Press: New York, 1963; Vol II, p 221.

Synthesis and Antiviral Activity of 9-Alkoxypurines. 2. 9-(2,3-Dihydroxypropoxy)-, 9-(3,4-Dihydroxybutoxy)-, and 9-(1,4-Dihydroxybut-2-oxy)purines

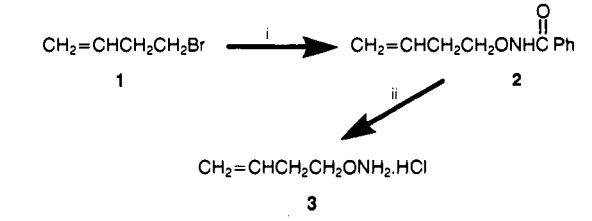
Stuart Bailey,* Michael R. Harnden, Richard L. Jarvest, Ann Parkin, and Malcolm R. Boyd

Beecham Pharmaceuticals Research Division, Biosciences Research Centre, Great Burgh, Yew Tree Bottom Road, Epsom, Surrey KT18 5XQ, U.K. *Received February 6, 1990*

Reaction of alkenoxyamines (**3**, **5**) or (*R*,*S*)-, (*R*)-, and (*S*)-hydroxy-protected derivatives of hydroxyalkoxyamines (**20a**,**b**, **37a-c**) with 4,6-dichloro-2,5-diformamidopyrimidine (**4**) and cyclization of the resultant 6-[(alkenoxy)amino]- and 6-(alkoxyamino)pyrimidines (**6**, **7**, **21a**,**b**, **38a**,**b**,**c**) by heating with diethoxymethyl acetate afforded 9-alkenoxy- and 9-alkoxy-6-chloropurines (**9**, **10**, **22a**,**b**, **39a-c**, **40a**). These were subsequently converted to 9-(2,3-dihydroxypropoxy), 9-(3,4-dihydroxybutoxy), and 9-(1,4-dihydroxybut-2-oxy) derivatives of guanine and 2-aminopurine (**13-16**, **25-28**, **41a-c**, **42a**). A 2-amino-6-methoxypurine derivative (**17**) was also prepared. The racemic guanine derivative **13** showed potent and selective activity against herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), but was less active against varicella zoster virus (VZV). Its antiviral activity is attributable to the *S* isomer (**28**), which was found to be more active than acyclovir against HSV-1 and HSV-2 and about 4 times less active than acyclovir against VZV. The S enantiomer of 9-(1,4-dihydroxybut-2-oxy)guanine (**41c**) also showed noteworthy antiviral activity in cell culture. Although this acyclonucleoside (**41c**) is only weakly active against HSV-1 and inactive against HSV-2, it is about twice as active as acyclovir against VZV.

In part 1 of this series of publications on novel 9-alkoxypurines,[1] the synthesis and antiviral activity of 9-(3-hydroxypropoxy)- and 9-[3-hydroxy-2-(hydroxymethyl)-propoxy]purines were reported. In this publication we describe the synthesis and antiviral properties of 9-(2,3-dihydroxypropoxy), 9-(3,4-dihydroxybutoxy), and 9-(1,4-dihydroxybut-2-oxy) derivatives of guanine and their stereoisomers. The latter compounds can be regarded as analogues of the antiviral acyclonucleosides (*R*)-9-(3,4-dihydroxybutyl)guanine (buciclovir),[2,3] (*S*)-9-[2,3-(dihydroxypropoxy)methyl]guanine [(*S*)-INDG][4] and 9-[4-hydroxy-2-(hydroxymethyl)butyl]guanine (2HM-HBG),[5,6]

Scheme I[a]



[a] (i) PhC(O)NHOH, NaH, DMF; (ii) concentrated HCl, EtOH, reflux, 3 h.

(1) Part 1: Harnden, M. R.; Wyatt, P. G.; Boyd, M. R.; Sutton, D. *J. Med. Chem.* **1990**, *33*, 187.
(2) Larsson, A.; Öberg, B.; Alenius, S.; Hagberg, C.-E.; Johansson, N.-G.; Lindborg, B.; Steyning, G. *Antimicrob. Agents Chemother.* **1983**, *23*, 664.
(3) Ericson, A.-C.; Larsson, A.; Aoki, F. Y.; Yisak, W.-A.; Johansson, N. G.; Öberg, B.; Datema, R. *Antimicrob. Agents Chemother.* **1985**, *27*, 753.
(4) Ashton, W. T.; Canning, L. F.; Reynolds, G. F.; Tolman, R. L.; Karkas, J. D.; Liou, R.; Davies, M.-E. M.; DeWitt, C. M.; Perry, H. C.; Field, A. K. *J. Med. Chem.* **1985**, *28*, 926.

0022-2623/91/1834-0057$02.50/0