

$\leq 2\theta \leq 140^\circ$) and the five-point method, monitoring one standard reflection every 50. Only 1430 reflections with $I > 2\sigma(I)$ [$\sigma(I)$ based on counting statistics] were considered as observed and used in the analysis. Seven low order reflections (0,2,0/-1,2,0/1,0,1/-2,0,1/-1,1,1/1,-1,1/-1,1) were discarded in the last cycle of refinement. Lorentz and polarization were applied but not absorption.

The structure was solved via direct methods and refined by full-matrix least squares²² with anisotropic and isotropic thermal parameters for non-hydrogen and hydrogen atoms respectively. All the hydrogens were located from difference Fourier map; H(41), H(42), H(51), and H(52) were constrained to ride on C(4) and C(5), respectively, during the last cycle of refinement. Final $R = 0.0853$, $R_w = 0.0869$, $w = [(F)^2 + 0.062F^2]^{-1}$ for 1430 reflections. The figures were obtained by using ORTEP²³ and PLUTO²⁴ whereas geometric calculations were performed with the PARST program.²⁵ All the calculations were performed using CRYSRULER.²⁶

Pharmacological Methods. Carrageenan-Induced Paw Edema. Ten or 20 female Wistar rats (200–250-g body weight) were used for each group. Hind paw volumes were measured using a water pletysmometer (Basile, Varese, Italy) according to the method described by Winter et al.²⁷ Then the compounds to be tested were administered intraperitoneally (ip) at a constant dose of 50 mg/kg, by using a solution in aqueous NaHCO_3 (pH 7.4) at a concentration of 10 mg/mL. Control rats received the

same volume of the vehicle. Thirty minutes later, 0.05 mL of 1% solution of carrageenan (Sigma, St. Louis, MO) was subcutaneously injected into the plantar surface of the right hind paw. The increase in paw volume 4 h after the injection of the phlogistic agent was adopted as a measure of the effect, and results were expressed as a percent reduction of control edema. Compounds showing 50% or more of inhibition (7q and 3) were further tested at 25 mg/kg following the procedure described above. Compounds showing 20–50% of inhibition (7a, 7d, 7e, 7m, and 7n) were tested at 100 mg/kg; for compounds (7d, 7m, and 7n) that showed no increase in activity at 100 mg/kg, a full dose-response curve starting from 25 mg/kg was also carried out. Approximate ID_{50} s were calculated by plotting the log of the dose of the compound versus inhibition of paw edema. Student's t test for grouped data was used for statistical evaluations. Differences were considered to be statistically significant when p was 0.05 or lower.

Acknowledgment. This work was supported in part by a grant from the Consiglio Nazionale delle Ricerche and the Ministero della Pubblica Istruzione.

Registry No. 1, 1553-60-2; 2, 22131-79-9; 4a, 127-06-0; 4b, 80606-74-2; 4c, 1192-28-5; 4d, 49805-38-1; 4e, 100-64-1; 4f, 1188-63-2; 4g, 1113-74-2; 4h, 4576-48-1; 4i, 80606-77-5; 4j, 4500-12-3; 4k, 49805-57-4; 4l, 30950-35-7; 4m, 10341-75-0; 4n, 23517-42-2; 4o, 574-66-3; 4p, 2157-52-0; 4q, 622-31-1; 4r, 622-32-2; 4s, 620-03-1; 4t, 38266-87-4; 5, 140-88-5; 6a, 119881-14-0; 6b, 125803-04-5; 6c, 119881-16-2; 6d, 125803-05-6; 6e, 119881-17-3; 6f, 125803-06-7; 6g, 103586-50-1; 6h, 125803-07-8; 6i, 125803-08-9; 6j, 125803-09-0; 6k, 125803-10-3; 6l, 125803-11-4; 6m, 103586-51-2; 6n, 125803-12-5; 6o, 125803-13-6; 6p, 119881-19-5; 6q, 103586-48-7; 6r, 125803-14-7; 6s, 125803-15-8; 6t, 125803-16-9; 7a, 103586-55-6; 7b, 125803-17-0; 7c, 103586-53-4; 7d, 125803-18-1; 7e, 103586-54-5; 7f, 103586-56-7; 7g, 103604-67-7; 7h, 103586-57-8; 7i, 125803-19-2; 7j, 125803-20-5; 7k, 125803-21-6; 7l, 125803-22-7; 7m, 103586-52-3; 7n, 125803-23-8; 7o, 15985-45-2; 7p, 103586-60-3; 7q, 103586-49-8; 7r, 103586-70-5; 7s, 103586-62-5; 7t, 103586-69-2.

Supplementary Material Available: Table IV giving final atomic coordinates and isotropic B equivalent, Table V giving anisotropic and isotropic thermal parameters, and Table VII giving cartesian coordinates of compounds 1, 2, and 7c (3 pages); Table VI giving observed and calculated structure factor amplitudes (8 pages). Ordering information is given on any current masthead page.

Retinobenzoic Acids. 5. Retinoidal Activities of Compounds Having a Trimethylsilyl or Trimethylgermyl Group(s) in Human Promyelocytic Leukemia Cells HL-60

Takeru Yamakawa, Hiroyuki Kagechika, Emiko Kawachi, Yuichi Hashimoto, and Koichi Shudo*

Faculty of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan.

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The retinoidal activities of trimethylsilyl or trimethylgermyl-containing retinobenzoic acids are discussed on the basis of differentiation-inducing activity on human promyelocytic leukemia cells HL-60. Compounds with a trimethylsilyl or trimethylgermyl group at the meta position of the generic formula 2 have more potent activities than the corresponding retinobenzoic acids with a *m*-tert-butyl group. Compounds having two *m*-trimethylsilyl or -trimethylgermyl groups also have strong activities, and (E)-4-[3-[3,5-bis(trimethylsilyl)phenyl]-3-oxo-1-propenyl]benzoic acid (22, Ch55S) and (E)-4-[3-[3,5-bis(trimethylgermyl)phenyl]-3-oxo-1-propenyl]benzoic acid (35, Ch55G) are more active than retinoic acid by 1 order of magnitude. However, in the para-substituted chalcone derivatives, the replacement of a *tert*-butyl group (49, Ch40) with a trimethylsilyl (27, Ch40S) or a trimethylgermyl (30, Ch40G) group caused the disappearance of the activity.

Retinoids are defined as "substances that elicit specific biological responses (that is, the specific activities of retinoic acid) through binding to the specific receptor(s)".^{1,2} Retinobenzoic acids are "a series of benzoic acid derivatives with potent retinoidal activities".³ They modulate the

cellular differentiation and proliferation in many types of cells in cases where retinoic acid (1, Chart I) acts as a modulator.^{2,4} Their mechanism of action seems to be the same as that of retinoic acid,^{5,6} and they also bind to the

- (1) Sporn, M. B.; Roberts, A. B.; Goodman, D. S., Eds. *The Retinoids*; Academic Press, Inc.: Orlando, FL, 1984.
- (2) Sporn, M. B.; Roberts, A. B.; Roche, N. S.; Kagechika, H.; Shudo, K. *J. Am. Acad. Dermatol.* 1986, 15, 756.

- (3) Shudo, K.; Kagechika, H. *Chemistry and Biology of Synthetic Retinoids*; Dawson, M. I., Okamura, W. H., Eds.; CRC Press: Boca Raton, FL, in press.
- (4) Jetten, A. M.; Anderson, K.; Deas, M. A.; Kagechika, H.; Lotan, R.; Rearick, J. I.; Shudo, K. *Cancer Res.* 1987, 47, 3523.

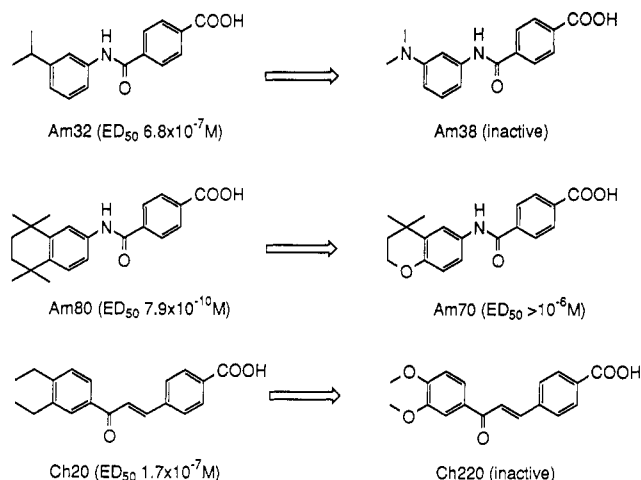
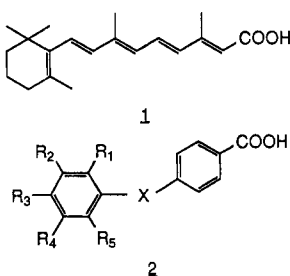


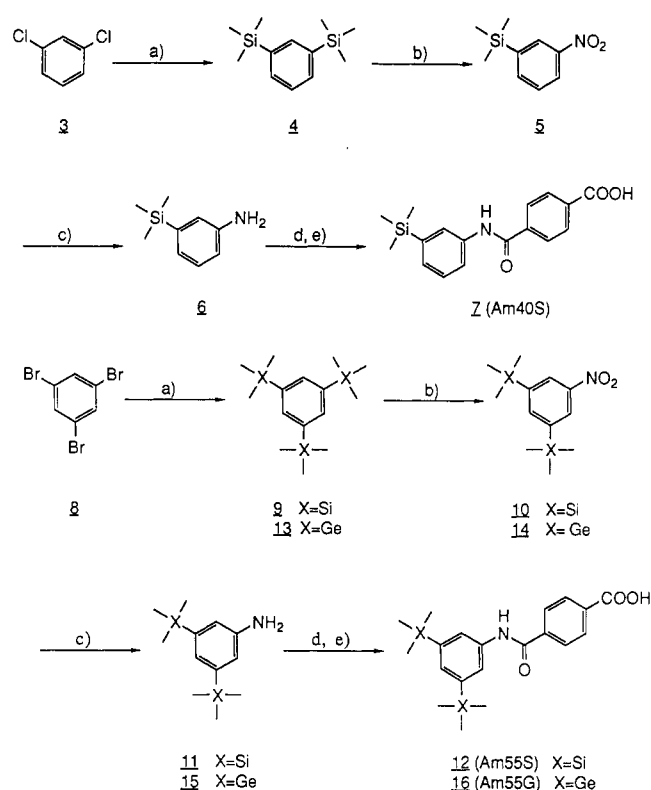
Figure 1. Diminished retinoidal activities caused by the introduction of polar atoms.

Chart I



same receptors.⁷⁻⁹ The generic chemical structure of retinobenzoic acids is represented by 2.¹⁰ Groups necessary for retinoidal activities are a bulky alkyl group, such as an isopropyl or a *tert*-butyl group, at the meta (R_2) position on one benzene ring and a carboxyl group at the para position of the other benzene ring. The linking group X can be varied (such as $-\text{NHCO}-$,^{11,12} $-\text{CONH}-$,^{12,13} $-\text{SO}_2\text{NH}-$, $-\text{COCH}=\text{CH}-$)^{14,15} $-\text{N}=\text{N}-$,^{16,17} and so forth), regardless of the electronic properties. It has a role in locating the two essential groups, the *m*-alkyl group and the *p*-carboxyl group, at the proper positions.¹⁸ Conse-

Scheme I^a



^a (a) TMSCl (or TMGCl)/ Mg ; (b) fuming $\text{HNO}_3/\text{Ac}_2\text{O}$; (c) $\text{H}_2/10\%$ Pd-C ; (d) $p\text{-ClCOC}_6\text{H}_4\text{COOCH}_3/\text{pyridine/benzene}$; (e) aqueous NaOH/EtOH .

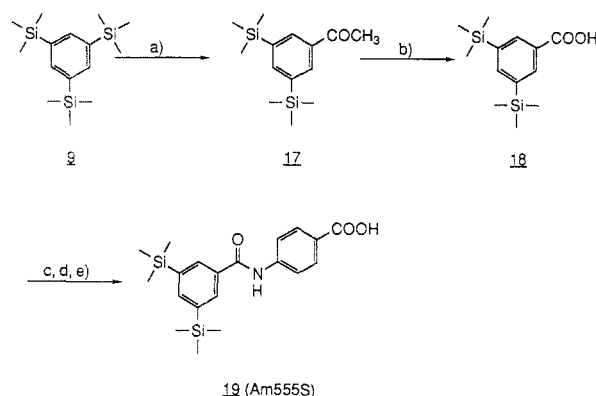
quently, the orientation and the distance between the *m*-benzylic methyl group and the carboxyl group are the most important factors for the activity.^{15,17,18} Contrary to the variability of the linking group X, the introduction of a polar atom, such as oxygen, sulfur, or nitrogen, into this alkyl group region causes remarkable diminution of the activity. Figure 1 shows that some compounds with a polar atom(s) instead of the benzylic carbon(s) have diminished abilities to induce differentiation of HL-60 cells.^{12,15} Thus, the hydrophobicity in this region (R_2 and R_3) is an important factor for potent retinoidal activities. In view of these structure-activity relationships of retinobenzoic acids, we thought that it would be very interesting to examine the effect of replacement of the benzylic carbon with a silicon or germanium atom, members of the same group (group IVB) of the periodic table. These atoms are tetravalent and have some hydrophobicity, but have different electronic and steric properties from the carbon atom. Therefore, silyl or germyl substitution is expected to have an effect on activity or pharmacokinetic behavior. In this study, the retinoidal activities of some benzoic acid derivatives with a trimethylsilyl (TMS) or trimethylgermyl (TMG) group(s) are discussed, on the basis of the ability to induce differentiation of human promyelocytic leukemia cell line HL-60 to mature granulocytes.

Chemistry

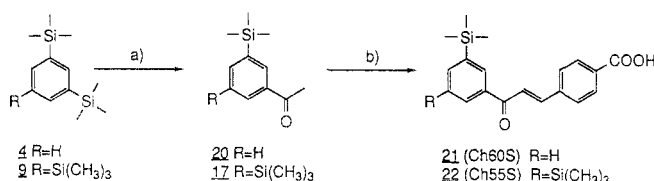
TMS- or TMG-substituted terephthalic monoanilides were synthesized as shown in Scheme I. Because nitration of (trimethylsilyl)benzene is known to result in a mixture of three regioisomers of nitro(trimethylsilyl)benzene and nitrobenzene, which is formed by ipso attack (nitrodesilylation),^{19,20} the method of selective nitrodesilylation of

- (5) Hashimoto, Y.; Kagechika, H.; Kawachi, E.; Shudo, K. *Chem. Pharm. Bull.* **1987**, *35*, 3190.
- (6) Takagi, K.; Suganuma, M.; Kagechika, H.; Shudo, K.; Nino-miya, M.; Muto, Y.; Fujiki, H. *J. Cancer Res. Clin. Oncol.* **1988**, *114*, 221.
- (7) Hashimoto, Y.; Kagechika, H.; Kawachi, E.; Shudo, K. *Jpn. J. Cancer Res.* **1988**, *79*, 473.
- (8) Kagechika, H.; Hashimoto, Y.; Kawachi, E.; Shudo, K. *Biochem. Biophys. Res. Commun.* **1988**, *155*, 503.
- (9) Hashimoto, Y.; Petkovich, M.; Gaub, M. P.; Kagechika, H.; Shudo, K.; Chambon, P. *Mol. Endocrinol.* **1989**, *3*, 1046.
- (10) Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Shudo, K. *Recent Advances in Chemotherapy, Anticancer Section*; Ishigami, J., Ed.; University of Tokyo Press: Tokyo, 1985; p 227.
- (11) Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Shudo, K. *Chem. Pharm. Bull.* **1984**, *32*, 4209.
- (12) Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Himi, T.; Shudo, K. *J. Med. Chem.* **1988**, *31*, 2182.
- (13) Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Shudo, K. *Chem. Pharm. Bull.* **1986**, *34*, 2275.
- (14) Shudo, K.; Kagechika, H.; Kawachi, E.; Hashimoto, Y. *Chem. Pharm. Bull.* **1985**, *33*, 404.
- (15) Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Shudo, K. *J. Med. Chem.* **1989**, *32*, 834.
- (16) Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Shudo, K. *Chem. Pharm. Bull.* **1985**, *33*, 5597.
- (17) Kagechika, H.; Himi, T.; Namikawa, K.; Kawachi, E.; Hashimoto, Y.; Shudo, K. *J. Med. Chem.* **1989**, *32*, 1098.
- (18) Kagechika, H.; Himi, T.; Kawachi, E.; Hashimoto, Y.; Shudo, K. *J. Med. Chem.* **1989**, *32*, 2292.

- (19) Colvin, E. W., Ed. *Silicon in Organic Synthesis*; Butterworths: London, 1981.

Scheme II^a

^a (a) $\text{AcCl}/\text{AlCl}_3/\text{CS}_2$; (b) $\text{Ca}(\text{OCl})_2/\text{K}_2\text{CO}_3/\text{KOH}/\text{H}_2\text{O}$; (c) $\text{SOCl}_2/\text{benzene}$; (d) $p\text{-NH}_2\text{C}_6\text{H}_4\text{COOCH}_3/\text{NEt}_3/\text{benzene}$; (e) aqueous NaOH/EtOH .

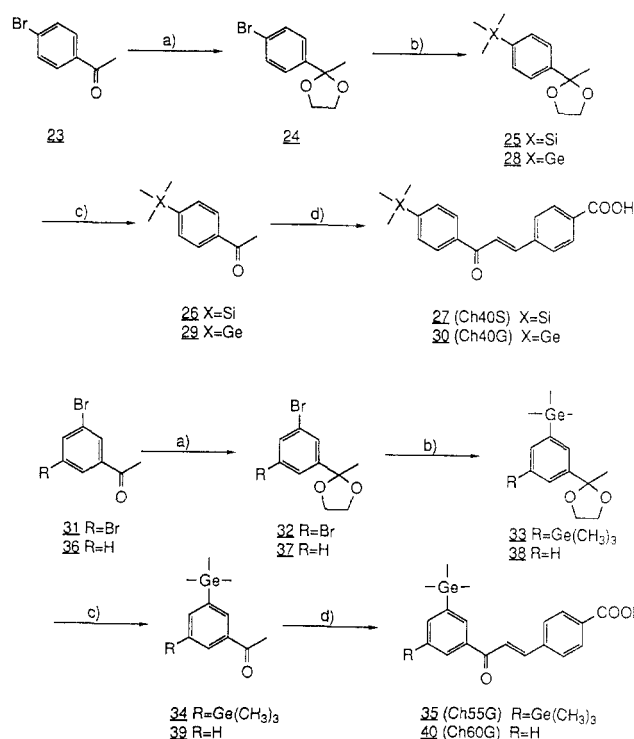
Scheme III^a

^a (a) $\text{AcCl}/\text{AlCl}_3/\text{CS}_2$; (b) $p\text{-HOCOC}_6\text{H}_4\text{COOCH}_3/\text{aqueous NaOH}/\text{THF}$.

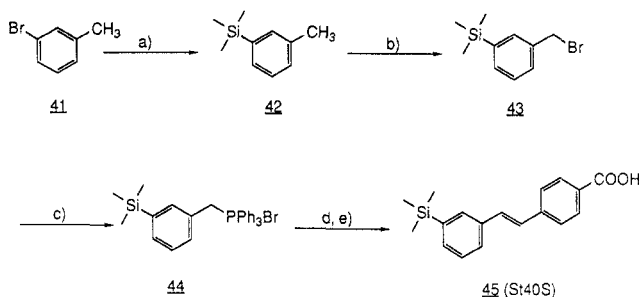
poly-trimethylsilylated benzene was employed.^{20,21} *m*-Bis(trimethylsilyl)benzene (4) was nitrated by fuming nitric acid in acetic anhydride to give *m*-nitro(trimethylsilyl)benzene (5) (50%). Similarly, *sym*-tris(trimethylsilyl)benzene (9) and *sym*-tris(trimethylgermyl)benzene (13) were nitrated to give 1-nitro-3,5-bis(trimethylsilyl)benzene (10) (59%) and 1-nitro-3,5-bis(trimethylgermyl)benzene (14) (42%), respectively. These nitro compounds were hydrogenated and condensed with a terephthalic acid moiety to give 7 (Am40S), 12 (Am55S), and 16 (Am55G).

Anilide derivative 19 (Am55S), which possesses a reversed amide group compared with 12, was synthesized by the acylsilylation^{19,20} (Scheme II). *sym*-Tris(trimethylsilyl)benzene (9) was acetylated by $\text{AcCl}-\text{AlCl}_3$ in CS_2 at 0 °C to give 3,5-bis(trimethylsilyl)acetophenone (17) (77%) and 3-(trimethylsilyl)acetophenone (20) (7%), which was formed by further protodesilylation. 3,5-Bis(trimethylsilyl)acetophenone (17) was converted to the corresponding substituted benzoic acid by the haloform reaction and then condensed with a *p*-aminobenzoic acid moiety to give 19.

Chalcone-4-carboxylic acids with a TMS or TMG group(s) were prepared by two synthetic methods. One is the selective acylsilylation described above (Scheme III). *m*-Bis(trimethylsilyl)benzene (4) was acetylated by $\text{AcCl}-\text{AlCl}_3$ in CS_2 at 0 °C to give 3-(trimethylsilyl)acetophenone (20) (44%) as a major product. 3-(Trimethylsilyl)acetophenone 20 was condensed with methyl terephthalaldehyde under basic aldol conditions to give 21 (Ch60S). Similarly, a bis(trimethylsilyl) derivative (22, Ch55S) was prepared by starting from *sym*-tris(trimethylsilyl)benzene (9). The other method for the synthesis of TMS or TMG-substituted acetophenones is tri-

Scheme IV^a

^a (a) $\text{HOCH}_2\text{CH}_2\text{OH}/p\text{-TsOH}/\text{benzene}$; (b) TMSCl (or TMGCl)/ Mg/THF ; (c) $\text{pyridine}\cdot\text{TsOH}/\text{acetone}/\text{H}_2\text{O}$; (d) $p\text{-HOCOC}_6\text{H}_4\text{COOCH}_3/\text{aqueous KOH}/\text{THF}$ ($i\text{-PrOH}$).

Scheme V^a

^a (a) Li/ether ; TMSCl ; (b) $\text{NBS}/\text{AIBN}/\text{CH}_2\text{Cl}_2$; (c) $\text{PPh}_3/\text{toluene}$; (d) $p\text{-HOCOC}_6\text{H}_4\text{COOCH}_3/\text{NaOCH}_3/\text{CH}_3\text{OH}$; (e) aqueous KOH/EtOH .

methylsilylation or trimethylgermylation of halogenated acetophenones whose carbonyl groups are protected (Scheme IV). *p*-Bromoacetophenone (23) was ketalized with ethylene glycol and then reacted with Mg and TMSCl to give 2-methyl-2-[4-(trimethylsilyl)phenyl]-1,3-dioxolane (25) (82%). Deprotection was carefully performed with pyridinium tosylate in quantitative yield. Deprotection using $\text{HCl}/\text{CH}_3\text{OH}$ or $\text{SiO}_2\cdot\text{H}_2\text{O}$ gave the protodesilylated compounds as byproducts. 4-(Trimethylsilyl)acetophenone (26) was condensed with methyl terephthalaldehyde under basic conditions to give 27 (Ch40S). Similarly, three corresponding TMG derivatives, 30 (Ch40G), 35 (Ch55G), and 40 (Ch60G), were synthesized from mono- or dibromoacetophenones (Scheme IV).

Stilbene-4-carboxylic acid (45, St40S) was synthesized from *m*-bromotoluene (41) by trimethylsilylation, followed by bromination, Wittig reaction with methyl terephthalaldehyde, and hydrolysis as shown in Scheme V.

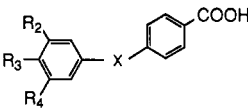
Biological Results

The ability to induce differentiation of human promyelocytic leukemia cell line HL-60 to mature granulo-

(20) Häbich, D.; Effenberger, F. *Synthesis* 1979, 841.

(21) Félix, G.; Dunoguès, J.; Calas, R. *Angew. Chem., Int. Ed. Engl.* 1979, 18, 402.

Table I. Differentiation-Inducing Activity of Trimethylsilyl- or Trimethylgermyl-Containing Retinoids



compound (code name)	linking group X	substituent			ED ₅₀ , M	relative activity ^a
		R ₂	R ₃	R ₄		
1 (retinoic acid)					2.4 × 10 ⁻⁹	100
7 (Am40S)	-NHCO-	TMS	H	H	8.5 × 10 ⁻⁸	2.4
46 (Am40)		tBu	H	H	7.0 × 10 ⁻⁷	0.32
12 (Am55S)		TMS	H	TMS	3.0 × 10 ⁻⁸	13
16 (Am55G)		TMG	H	TMG	4.2 × 10 ⁻⁸	15
47 (Am55)		tBu	H	tBu	3.6 × 10 ⁻⁸	15
19 (Am555S)	-CONH-	TMS	H	TMS	9.2 × 10 ⁻⁹	52.7
48 (Am555)		tBu	H	tBu	4.8 × 10 ⁻⁸	5.6
27 (Ch40S)	-COCH=CH-	H	TMS	H	inactive at 10 ⁻⁶	
30 (Ch40G)		H	TMG	H	inactive at 10 ⁻⁶	
49 (Ch40)		H	tBu	H	2.8 × 10 ⁻⁸	3.9
21 (Ch60S)		TMS	H	H	4.4 × 10 ⁻⁸	1.8
40 (Ch60G)		TMG	H	H	4.0 × 10 ⁻⁸	4.5
51 (Ch60)		tBu	H	H	1.6 × 10 ⁻⁷	0.81
22 (Ch55S)		TMS	H	TMS	1.4 × 10 ⁻¹⁰	860
35 (Ch55G)		TMG	H	TMG	2.1 × 10 ⁻¹⁰	1000
50 (Ch55)		tBu	H	tBu	2.1 × 10 ⁻¹⁰	640
45 (St40S)	-CH=CH-	TMS	H	H	1.0 × 10 ⁻⁹	85
52 (St40)		tBu	H	H	1.0 × 10 ⁻⁸	13

^a Relative activity was defined as the ratio of ED₅₀ (retinoic acid) to ED₅₀ (a test compound), both values having been obtained in concurrent experiments, multiplied by 100. Therefore, this is not the simple ratio of the ED₅₀ values shown in the table. The values shown are means of the ratios, when more than three repetitions were done, or representative ones (values for compounds 7 and 19).

cytes^{22,23} was examined as a measure of retinoidal activity. This activity of retinoids correlates well with other retinoidal activities.¹ The morphological changes were examined after Wright-Giemsa staining, and the nitro blue tetrazolium (NBT) reduction assay was employed as a functional marker of differentiation.²⁴ These two indexes of differentiation correlated well.^{11,13,14} Experiments were repeated at least twice, covering 5 magnitudes in concentration. The ED₅₀ values of active compounds were calculated from the NBT reduction assay data. Relative activity was defined as the ratio of the ED₅₀ of retinoic acid to the ED₅₀ of a test compound, both values having been obtained in concurrent experiments. ED₅₀ values and relative activities shown in Table I are representative ones or means.

The table shows the results of the differentiation-inducing activities of TMS or TMG-containing compounds, compared with the corresponding retinobenzoic acids having a *tert*-butyl (*t*Bu) group(s). Most of the silyl and germlyl analogues of retinobenzoic acids have potent activities on HL-60 cells. Analogue 7, the amide derivative with one *m*-TMS group, is more active than the corresponding *t*Bu derivative 46 by 1 order of magnitude. The introduction of the second *m*-*t*Bu group enhances the activity (46 vs 47). In the case of TMS-containing analogues, similarly, *m,m*-disubstituted compound 12 is more active than 7 and ¹/₇ as active as retinoic acid. Analogue 16, having two *m*-TMG groups, is also as active as the corresponding compounds 47 and 12. There appears to be no different effect on activity with the three kinds of benzylic atom (C, Si, or Ge) in this series. In contrast, silyl analogue 19 has more potent differentiation-inducing activity than the corresponding carbon analogue 48.

In the chalcone series, the derivatives with either one or two meta substituents have differentiation-inducing potencies. TMS-substituted compound 21 and TMG-substituted compound 40 are as active as or slightly more potent than the corresponding retinobenzoic acid with a *t*Bu group (51). However, the silyl or germlyl analogue (27 or 30) is inactive below 10⁻⁶ M in the HL-60 assay, while *t*Bu congener 49 is active at 10⁻⁸ M (and more active than its meta isomer 51). In the *t*Bu-containing chalcone series, para substitution is preferable, and this is exceptional throughout the structure-activity relationships for retinobenzoic acids.¹⁷ In this case, also, the second meta substituent enhances activity. Thus, 22 and 35, like 50 (Ch55), are more active than retinoic acid by approximately 1 order of magnitude.

Finally, 3'-(trimethylsilyl)stilbene-4-carboxylic acid (45) was found to be 6.5 times as active as 52, which has a *m*-*t*Bu group. This result is similar to the case in the amide compounds (7 vs 46).

Discussion

A number of organosilicon derivatives of bioactive compounds have been synthesized and their activities have been investigated.²⁵ Such silyl-containing analogues could be classified roughly into two groups. One is silylated derivatives of known compounds, most of which are O- or N- (or occasionally C-) trimethylsilylated derivatives, useful mainly as prodrugs. The other group is organosilicon compounds having analogous structures to known bioactive carbon compounds, in which tetravalent silicon and germanium (though there are few examples of effective germanium analogues of drugs) are bioisosters of carbon.

In the monosubstituted retinobenzoic acids, generally, a TMS or TMG group at a meta position enhances activity more than a *t*Bu group. Thus, the low activities of 46 (Am40), 51 (Ch60), and 52 (St40) were increased by in-

(22) Collins, S. J.; Gallo, R. C.; Gallagher, R. E. *Nature (London)* 1977, 270, 347.

(23) Koeffler, H. P. *Blood* 1983, 62, 709.

(24) Collins, S. J.; Ruscetti, F. W.; Gallagher, R. E.; Gallo, R. C. *J. Exp. Med.* 1979, 149, 969.

(25) Tacke, R.; Wannagat, U. *Topics in Current Chemistry*; Dewar, M.J.S., Ed.; Springer-Verlag: Berlin, 1979; Vol. 84, p 1.

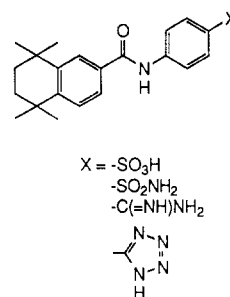
introduction of a silicon or germanium atom. In the case of the disubstituted compounds, the activities are nearly the same, regardless of the kind of benzylic atom. The only case where bis substitution (with TMS) led to an increase in activity over the carbon analogue was that involving 19 (Am555S) and 48 (Am555). Bis(trimethylsilyl) compound 22 (Ch55S) and bis(trimethylgermyl) compound 35 (Ch55G), which correspond to one of the most potent retinobenzoic acids, the di-*tert*-butyl compound 50 (Ch55), also have the highest activities and are more active than retinoic acid by 1 order of magnitude. In this case the introduction of silyl or germly groups did not enhance the activity over that of the *t*Bu analogue, probably because the effective concentration of around 10^{-10} M is already so extremely low.

The increased activities may be partially due to the different chemical character of the silicon or germanium atom from that of the carbon atom. First, since the C-Si or C-Ge bond is longer than the C-C bond, the hydrophobic groups are sterically bulkier than the *t*Bu group. Thus, the volume of the hydrophobic region in the binding site of the receptor is somewhat larger than the volume of the *t*Bu group. This is also supported by the recent observation by Shroot et al. that some compounds possessing an adamantyl or 1-methylcyclohexyl group as the meta hydrophobic group of 2 had potent retinoidal activities.²⁶ Second, the silicon or germanium atom is more electropositive than the carbon atom. Therefore, the surface electronic potentials of the methyl groups attached to these atoms are more negative than those of the *t*Bu group or other groups of the compounds shown in Figure 1. It is an important finding that the significant benzylic carbon atom for the retinoidal activities or for the binding to the specific receptor can be replaced with a silicon or germanium atom.

The only exception was in the *para*-substituted chalcone derivatives, where the TMS (27, Ch40S) or TMG (30 Ch40G) group failed to give differentiation-inducing activity, although 49 (Ch40) with a *p*-*t*Bu group conferred the activity. At present, we do not know whether this is due to the chemical characteristics resulting in a different affinity to the receptor from that of the carbon analogue or due to different pharmacokinetic-metabolic behavior.

Regarding the structure-activity relationships of retinobenzoic acids, the general formula (2) consists of three parts: hydrophobic alkylbenzene, the linking group X, and benzoic acid. From our previous studies,^{12,15,17} the linking group X can be varied with retention of high and specific retinoidal activities, and so the chemical and physical properties of retinoids can vary widely. The result in this study showed that the carbon skeleton in the hydrophobic region is not essential and can be replaced with other hydrophobic groups. With respect to the last part of retinobenzoic acids, the benzoic acid moiety, or a functional group that can be a precursor of a carboxyl group (ester, amide, and other groups, which can be metabolized to the carboxylic acid), can be effective, although the activity is reduced by 1 or 2 orders of magnitude.^{12,15} However, compounds which possess a sulfo, aminosulfonyl, amidino, or tetrazolyl group, instead of a carboxyl group (Chart II), are inactive or have very weak activity on HL-60 cells (data not shown). Such bioisosteric conversion of the terminal polar group was not successful, and so the carboxyl group (or groups metabolically convertible to the carboxylic acid) is the only essential group in the structure of retinobenzoic

Chart II



acids for the induction of differentiation of HL-60 cells.

In conclusion, some trimethylsilyl or trimethylgermyl-containing benzoic acid derivatives are potent differentiation-inducers of HL-60 cells and should be included in the category of "retinoids". Since the physicochemical properties or pharmacological behavior of these compounds should be very different from those of conventional retinoids, they may be useful candidates for clinical applications as retinoids in oncology, dermatology, and immunology.

Experimental Section

Cells and Culture. The human promyelocytic leukemia cells HL-60 were provided by Prof. F. Takaku (Faculty of Medicine, University of Tokyo) and have been maintained in continuous suspension culture. The cells were cultured in plastic flasks in RPMI1640 medium, supplemented with 5% fetal calf serum (FCS) and antibiotics (penicillin G and streptomycin), at 37 °C in a humidified atmosphere of 5% CO₂ in air.

Test compounds were dissolved in ethanol at 0.2 mM and added to the cells, which were seeded at about 8×10^4 cells/mL; the final ethanol concentration was kept below 0.5%. Control cells were given only the same volume of ethanol. Retinoic acid, a positive control, was always assayed at the same time. The cells were incubated for 4 days and stained with Wright-Giemsa. Differential counts were then performed under a light microscope on a minimum of 200 cells. Nitro blue tetrazolium (NBT) reduction was assayed as described.²⁴ Cells were incubated for 20 min at 37 °C in RPMI1640 medium (5% FCS) and an equal volume of phosphate-buffered saline (PBS) containing NBT (0.2%) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA; 200 ng/mL). The percentage of cells containing blue-black formazan was determined on a minimum of 200 cells. The results of these two evaluations were always in good agreement.

The assays of test compounds were performed at least twice. ED₅₀ values of active compounds were calculated from the NBT reduction assay data by means of the van der Waerden method. Relative activities were calculated as the ratio of the ED₅₀ of retinoic acid to the ED₅₀ of the test compound obtained in concurrent experiments, multiplied by 100.

Chemistry. Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. Elemental analyses were carried out in the Microanalytical Laboratory, Faculty of Pharmaceutical Sciences, University of Tokyo, and were within $\pm 0.4\%$ of the theoretical values. NMR spectra were recorded on JEOL FX 100-MHz and JEOL GX 400-MHz NMR spectrometers. Chemical shifts are expressed in ppm relative to tetramethylsilane. Compounds with *tert*-butyl group(s) (compounds 46-52) were prepared as described previously.^{12,15,17}

4-[[3-(Trimethylsilyl)phenyl]carbamoyl]benzoic Acid (7, Am40S). A solution of *m*-dichlorobenzene (3, 15.0 g, 0.10 mol) in 30 mL of HMPA was added to a mixture of freshly distilled chlorotrimethylsilane (32.4 g, 0.30 mol) and Mg powder (6.0 g, 0.25 mol) in 60 mL of HMPA under Ar and the mixture was refluxed for 61 h.²⁷ The mixture was poured into ice water and was extracted with ether/*n*-hexane (2:1). The organic layer was washed with H₂O and brine and dried over Na₂SO₄. The crude

(26) Darmon, M.; Rocher, M.; Cavey, M.-T.; Martin, B.; Rabilloud, T.; Delescluse, C.; Shroot, B. *Skin Pharm.* 1988, 1, 161.

(27) Bourgeois, P.; Calas, R.; Jousseau, E.; Gervat, J. *J. Organomet. Chem.* 1975, 84, 165.

mixture was distilled (bp_{3mmHg} 87–88 °C) to give *m*-bis(trimethylsilyl)benzene (4). A solution of 1.50 g (6.74 mmol) of 4 in 4 mL of Ac₂O was heated at 130 °C.²⁸ To this solution was added 1.6 mL of fuming nitric acid (94%; 36 mmol) in 5 mL of Ac₂O portionwise over 30 min, and stirring was continued for another 30 min. After cooling, the mixture was poured into ice-cooled 2% K₂CO₃ and extracted with CH₂Cl₂. The organic layer was washed successively with H₂O, 2% KOH (twice), H₂O, and brine and dried over Na₂SO₄. The crude mixture was chromatographed on silica gel to give *m*-nitro(trimethylsilyl)benzene (5, 50%), 1-nitro-2,4-bis(trimethylsilyl)benzene (15%), and 1,3-dinitro-5-trimethylsilylbenzene (2%). Compound 5 (200 mg, 1.02 mmol) was hydrogenated over 10% Pd–C (38 mg) in 7.5 mL of benzene.²¹ The crude product was purified by silica gel column chromatography to give *m*-(trimethylsilyl)aniline (6, quant. yield). Compound 6 (135 mg, 0.87 mmol) was condensed with terephthalic acid monomethyl ester chloride (179 mg, 0.90 mmol) in 8 mL of dry benzene and 1 mL of pyridine. The mixture was poured into H₂O and extracted with AcOEt. The organic layer was washed successively with 0.2 M Cu(NO₃)₂, H₂O, 1 N NaHCO₃, H₂O, and brine and dried over Na₂SO₄. The crude mixture was chromatographed on silica gel to give methyl 4-[[3-(trimethylsilyl)phenyl]carbamoyl]benzoate (Am41S, quant. yield), which was hydrolyzed (aqueous NaOH–EtOH) to give 7 (Am40S, 61%). **Am41S**: colorless needles (from CH₂Cl₂–*n*-hexane); mp 125–126 °C; ¹H NMR (100 MHz, CDCl₃) δ 0.28 (s, 9 H), 3.96 (s, 3 H), 7.3–7.8 (m, 5 H), 7.93 (d, 2 H, *J* = 8 Hz), 8.13 (d, 2 H, *J* = 8 Hz); ¹³C NMR (25 MHz, CDCl₃) δ –1.25 (q), 52.2 (q), 121.3 (d), 125.3 (d), 127.1 (d), 128.1 (d), 129.4 (d), 129.6 (d), 132.2 (s), 137.2 (s), 138.7 (s), 141.2 (s), 165.2 (s), 165.9 (s). 7 (**Am40S**): colorless prisms (from CH₃OH); mp 211–213 °C; ¹H NMR (100 MHz, CDCl₃–DMSO-*d*₆) δ 0.29 (s, 9 H), 7.3–7.8 (m, 4 H), 8.05 (d, 2 H, *J* = 8 Hz), 8.13 (d, 2 H, *J* = 8 Hz), 9.66 (br s, 1 H). Anal. (C₁₇H₁₉NO₃Si·1/4 H₂O) C, H, N.

4-[[3,5-Bis(trimethylsilyl)phenyl]carbamoyl]benzoic Acid (12, Am55S). A solution of *sym*-tribromobenzene (8, 12.5 g, 39.7 mmol) in 40 mL of THF was added slowly to a mixture of freshly distilled chlorotrimethylsilane (15.25 g, 140 mmol) and Mg powder (3.65 g, 150 mmol) in 15 mL of THF under Ar and the mixture was refluxed overnight. The mixture was filtered and the precipitate was washed with ether and CH₂Cl₂. The organic layer was washed with H₂O and dried over Na₂SO₄. The crude mixture was chromatographed on silica gel to give *sym*-tris(trimethylsilyl)benzene (9, 56%) and 3,3',5,5'-tetrakis(trimethylsilyl)biphenyl (8%). A solution of 0.4 mL of fuming nitric acid (94%, 9.1 mmol) in 3 mL of Ac₂O was added to a solution of 9 (1.18 g, 4.00 mmol) in 2 mL of Ac₂O at –10 °C and the mixture was stirred for 2 h and then at room temperature overnight. The mixture was poured into ice-cooled 0.1 N NaOH and extracted with CH₂Cl₂. The organic layer was washed with H₂O and dried over Na₂SO₄. After removal of the solvent, the residue was chromatographed on silica gel to give 1-nitro-3,5-bis(trimethylsilyl)benzene (10, 59%) and 2-nitro-1,3,5-tris(trimethylsilyl)benzene (16%). Compound 10 (264 mg, 0.99 mmol) was hydrogenated on 10% Pd–C (27 mg) in 15 mL of benzene. The crude product was purified by silica gel column chromatography to give 3,5-bis(trimethylsilyl)aniline (11, 96%). Compound 11 (220 mg, 0.93 mmol) was condensed with terephthalic acid monomethyl ester chloride (187 mg, 0.94 mmol) in 10 mL of dry benzene and 1 mL of pyridine. The mixture was poured into H₂O and extracted with AcOEt. The organic layer was washed successively with 0.2 M Cu(NO₃)₂, H₂O, 1 N NaHCO₃ and H₂O, and dried over Na₂SO₄. The crude product was recrystallized to give methyl 4-[[3,5-bis(trimethylsilyl)phenyl]carbamoyl]benzoate (Am56S, 95%), which was hydrolyzed (aqueous NaOH–EtOH) to give 12 (Am55S, 88%). **Am56S**: colorless prisms (from CH₂Cl₂–*n*-hexane); mp 212.5–213.5 °C; ¹H NMR (400 MHz, CDCl₃) δ 0.30 (s, 18 H), 3.97 (s, 3 H), 7.46 (t, 1 H, *J* = 1 Hz), 7.76 (d, 2 H, *J* = 1 Hz), 7.79 (br s, 1 H), 7.96 (d, 2 H, *J* = 8 Hz), 8.17 (d, 2 H, *J* = 8 Hz). 12 (**Am55S**): colorless needles (from AcOEt–*n*-hexane); mp 252–253.5 °C; ¹H NMR (400 MHz, CDCl₃) δ 0.30 (s, 18 H), 7.46 (t, 1 H, *J* = 1 Hz), 7.77 (br s, 2 H), 7.80 (br s, 1 H), 7.99 (d, 2 H, *J* = 8 Hz), 8.21 (d, 2 H, *J* = 8 Hz). Anal. (C₂₀H₂₇NO₃Si₂) C, H, N.

4-[[3,5-Bis(trimethylgermyl)phenyl]carbamoyl]benzoic Acid (16, Am55G). Chlorotrimethylgermane (1.39 g, 9.07 mmol) and *sym*-tribromobenzene (8, 837 mg, 2.66 mmol) were added to a mixture of Mg (216 mg, 8.88 mmol) in 10 mL of THF under Ar and the mixture was refluxed for 5 h and then stirred at room temperature for 15 h. The mixture was diluted with petroleum ether and filtered. The precipitates was washed with petroleum ether and the filtrate was evaporated to give a pale-yellow oil. The crude mixture was chromatographed on silica gel and then distilled under vacuum (bp_{1mmHg} 90 °C) to give *sym*-tris(trimethylgermyl)benzene (13, 48%). A solution of 0.08 mL of fuming nitric acid (94%, 1.8 mmol) in 3 mL of Ac₂O was added to a solution of 13 (500 mg, 1.17 mmol) in 10 mL of Ac₂O at –10 °C and the mixture was stirred for 3 h. The mixture was poured into ice-cooled 1 N Na₂CO₃ and extracted with CH₂Cl₂. The organic layer was washed with H₂O and brine, and dried over Na₂SO₄. After removal of the solvent, the residue was chromatographed on silica gel to give 1-nitro-3,5-bis(trimethylgermyl)benzene (14, 42%). Compound 14 (125 mg, 0.35 mmol) was hydrogenated on 10% Pd–C (15 mg) in 10 mL of EtOH. The crude product was purified by silica gel column chromatography to give 3,5-bis(trimethylgermyl)aniline (15, 94%). Compound 15 (108 mg, 0.33 mmol) was condensed with terephthalic acid monomethyl ester chloride (66 mg, 0.33 mmol) in 10 mL of dry benzene and 1 mL of pyridine. The mixture was poured into 2 N HCl and extracted with CH₂Cl₂. The organic layer was washed successively with H₂O, 0.2 M Cu(NO₃)₂, H₂O, and brine, and dried over Na₂SO₄. The crude product was chromatographed on silica gel to give methyl 4-[[3,5-bis(trimethylgermyl)phenyl]carbamoyl]benzoate (Am56G, 68%), which was hydrolyzed (aqueous NaOH–EtOH) to give 16 (Am55G, 82%). **Am56G**: colorless prisms; ¹H NMR (400 MHz, CDCl₃) δ 0.42 (s, 18 H), 3.97 (s, 3 H), 7.36 (t, 1 H, *J* = 1 Hz), 7.70 (br s, 2 H), 7.78 (br s, 1 H), 7.96 (d, 2 H, *J* = 8 Hz), 8.17 (d, 2 H, *J* = 8 Hz). 16 (**Am55G**): colorless prisms (from EtOH–*n*-hexane) mp 233–234 °C; ¹H NMR (400 MHz, CDCl₃) δ 0.42 (s, 18 H), 7.35 (br s, 1 H), 7.75 (br s, 2 H), 7.97 (d, 2 H, *J* = 8 Hz), 8.16 (d, 2 H, *J* = 8 Hz). Anal. (C₂₀H₂₇NO₃Ge₂) C, H, N.

4-[[[3,5-Bis(trimethylsilyl)phenyl]carbonyl]amino]benzoic Acid (19, Am555S). A solution of AcCl (130 mg, 1.66 mmol) in 5 mL of CS₂ (freshly distilled from P₂O₅) was added slowly to a mixture of *sym*-tris(trimethylsilyl)benzene (9, 500 mg, 1.70 mmol) and AlCl₃ (217 mg, 1.63 mmol) in 10 mL of CS₂ at 0 °C and the mixture was stirred for 1 h. After removal of the solvent, 1 N NaHCO₃ was added and the mixture was extracted with CH₂Cl₂. The organic layer was washed with H₂O and dried over MgSO₄. The crude mixture was chromatographed on silica gel to give 3,5-bis(trimethylsilyl)acetophenone (17, 77%), 3-(trimethylsilyl)acetophenone (20, 7%) and 9 (15%). A suspension of Ca(OCl)₂ (500 mg, 3.50 mmol), K₂CO₃ (345 mg, 2.50 mmol), and KOH (85%, 100 mg, 1.49 mmol) in 10 mL of H₂O was vigorously shaken at 65 °C for 30 min and then filtered.²⁹ Compound 17 (128 mg, 1.00 mmol) was added to the filtrate and the mixture was heated at 110 °C for 7.5 h. After cooling, NaHSO₃ (10 mg) was added and the mixture was extracted with AcOEt. The organic layer was washed with H₂O and brine and dried over Na₂SO₄. The crude mixture was chromatographed on silica gel to give 3,5-bis(trimethylsilyl)benzoic acid (18, 33%) and 17 (51%). A mixture of 18 (41 mg, 0.15 mmol), CaCO₃ (40 mg), SOCl₂ (40 mg, 0.34 mmol) and a drop of DMF in 5 mL of dry benzene was stirred at room temperature for 2 h. After filtration and removal of the solvent, the residue was dissolved in 5 mL of dry benzene. To this solution were added methyl 4-aminobenzoate (30 mg, 0.20 mmol) and 0.5 mL of triethylamine, and the mixture was stirred overnight. The mixture was poured into 0.5 N HCl and extracted with CH₂Cl₂. The organic layer was washed with H₂O and brine and dried over Na₂SO₄. The crude mixture was chromatographed on silica gel to give methyl 4-[[3,5-bis(trimethylsilyl)phenyl]carboxamido]benzoate (Am556S, 36%), which was hydrolyzed (aqueous NaOH–EtOH) to give 19 (Am555S, 66%). 19 (**Am555S**): colorless prisms (from AcOEt–*n*-hexane), mp 280 °C dec; ¹H NMR (400 MHz, CDCl₃) δ 0.33 (s, 18 H), 7.80 (d, 2 H, *J* = 8.5 Hz), 7.85 (br s, 1 H), 7.92 (br s, 1 H), 7.95 (br s, 2 H), 8.13 (d, 2 H, *J* = 8.5

(28) Hashimoto, T. *Yakugaku Zasshi* 1967, 87, 524.(29) Blatt, A. H., Ed. *Organic Syntheses*; Wiley: New York, 1966; Collect. Vol. II, 428.

Hz). Anal. ($C_{20}H_{27}NO_3Si_2$) C, H, N.

(E)-4-[3-Oxo-3-[3-(trimethylsilyl)phenyl]-1-propenyl]benzoic Acid (21, Ch60S). A solution of AcCl (147 mg, 1.87 mmol) in 3 mL of CS_2 (freshly distilled from P_2O_5) was added slowly to a mixture of *m*-bis(trimethylsilyl)benzene (4, 419 mg, 1.88 mmol) and $AlCl_3$ (250 mg, 1.87 mmol) in 10 mL of CS_2 and the mixture was stirred for 5.5 h. After removal of the solvent, 1 N $NaHCO_3$ was added and the whole was extracted with CH_2Cl_2 . The organic layer was washed with H_2O and dried over $MgSO_4$. The crude mixture was chromatographed on silica gel to give 3-(trimethylsilyl)acetophenone (20, 44%). To a solution of 20 (130 mg, 0.68 mmol) and methyl terephthalaldehyde (127 mg, 0.77 mmol) in 5 mL of THF was added 3 mL of 1 N NaOH and the mixture was stirred overnight. The mixture was poured into 0.5 N HCl and extracted with AcOEt. The organic layer was washed with H_2O and dried over Na_2SO_4 . The crude mixture was chromatographed on silica gel to give 21 (30%). 21: pale-yellow needles (from CH_2Cl_2 -*n*-hexane); mp 179–180 °C; 1H NMR (400 MHz, $CDCl_3$) δ 0.33 (s, 9 H), 7.51 (t, 1 H, $J = 8$ Hz), 7.62 (d, 1 H, $J = 16$ Hz), 7.74 (d, 2 H, $J = 8$ Hz), 7.76 (d, 1 H, $J = 8$ Hz), 7.82 (d, 1 H, $J = 16$ Hz), 7.99 (d, 1 H, $J = 8$ Hz), 8.14 (d, 2 H, $J = 8$ Hz), 8.15 (m, 1 H). Anal. ($C_{19}H_{20}O_3Si$) C, H.

(E)-4-[3-[3,5-Bis(trimethylsilyl)phenyl]-3-oxo-1-propenyl]benzoic Acid (22, Ch55S). 3,5-Bis(trimethylsilyl)acetophenone (17) and methyl terephthalaldehyde were condensed according to the method described for 21 to give 22 (66%). 22: pale-yellow prisms (from AcOEt-*n*-hexane); mp 194–195.5 °C; 1H NMR (400 MHz, $CDCl_3$) δ 0.33 (s, 18 H), 7.59 (d, 1 H, $J = 16$ Hz), 7.73 (d, 2 H, $J = 8$ Hz), 7.80 (d, 1 H, $J = 16$ Hz), 7.87 (t, 1 H, $J = 1$ Hz), 8.10 (d, 2 H, $J = 1$ Hz), 8.16 (d, 2 H, $J = 8$ Hz). Anal. ($C_{22}H_{26}O_3Si_2$) C, H.

(E)-4-[3-Oxo-3-[4-(trimethylsilyl)phenyl]-1-propenyl]benzoic Acid (27, Ch40S). A mixture of 4-bromoacetophenone (23, 4.98 g, 25.0 mmol), ethylene glycol (2.33 g, 37.5 mmol), and *p*-TsOH· H_2O (238 mg, 1.25 mmol) in 180 mL of dry benzene was heated at reflux in a flask equipped with a Dean-Stark apparatus. After 8 h, ethylene glycol (1.55 g, 25 mmol) was added and the mixture was refluxed for 31 h. The mixture was washed with 1 N $NaHCO_3$, H_2O , and brine and dried over Na_2SO_4 . 2-(4-Bromophenyl)-2-methyl-1,3-dioxolane (24) was obtained as colorless prisms (quant. yield). A mixture of Mg powder (250 mg, 10.3 mmol) and CH_3I (20 mg) was heated at reflux in 15 mL of freshly distilled THF under Ar for 1.5 h. A solution of 24 (2.11 g, 8.68 mmol) in 20 mL of THF was added and the mixture was heated at reflux for 1 h. Chlorotrimethylsilane (1.10 g, 10.1 mmol; freshly distilled) was added at 55 °C and the mixture was heated at reflux. After 4 h, chlorotrimethylsilane (0.30 g, 2.76 mmol) was added again, the refluxing was continued for 1.5 h, and then the mixture was stirred overnight at room temperature. The mixture was diluted with ether, washed with H_2O , 1 N HCl, and H_2O , and dried over Na_2SO_4 . The crude product was chromatographed on silica gel to give 2-methyl-2-[4-(trimethylsilyl)phenyl]-1,3-dioxolane (25, 82%) as pale-yellow crystals (mp 59 °C). 25 (1.23 g, 5.20 mmol) and pyridinium tosylate (197 mg, 0.78 mmol) were dissolved in 30 mL of acetone and 4.7 mL of H_2O and the mixture was heated at reflux for 2.5 h and then stirred overnight at room temperature.³⁰ The solvent was removed and the residue was diluted with ether. The organic layer was washed successively with 1 N Na_2CO_3 , H_2O , 0.2 M $CuSO_4$, and H_2O and dried over Na_2SO_4 . The solvent was removed to give 4-(trimethylsilyl)acetophenone (26, 99%) as a yellow oil. Compound 26 was condensed with methyl terephthalaldehyde according to the method described for 21 to give 27 (30%). 27: pale-yellow prisms (from CH_3OH); mp 236.5–238.5 °C; 1H NMR (400 MHz, $CDCl_3$) δ 0.32 (s, 9 H), 7.61 (d, 1 H, $J = 16$ Hz), 7.68 (d, 2 H, $J = 8$ Hz), 7.73 (d, 2 H, $J = 8$ Hz), 7.82 (d, 1 H, $J = 16$ Hz), 7.99 (d, 2 H, $J = 8$ Hz), 8.14 (d, 2 H, $J = 8$ Hz). Anal. ($C_{19}H_{20}O_3Si$) C, H.

(E)-4-[3-Oxo-3-[4-(trimethylgermyl)phenyl]-1-propenyl]benzoic Acid (30, Ch40G). A solution of 2-(4-bromophenyl)-2-methyl-1,3-dioxolane (24, 972 mg, 4.00 mmol) in 12 mL of THF was added to a mixture of Mg turnings (108 mg, 4.44 mmol) and chlorotrimethylgermane (766 mg, 5.00 mmol), and the mixture was heated at reflux for 1.5 h and then stirred

overnight at room temperature. The mixture was diluted with petroleum ether and filtered. The filtrate was evaporated and chromatographed on silica gel to give 2-methyl-2-[4-(trimethylgermyl)phenyl]-1,3-dioxolane (28, 83%) as colorless prisms (mp 63.5–65 °C). Compound 28 (420 mg, 1.50 mmol) and pyridinium tosylate (56 mg, 0.22 mmol) were dissolved in 10 mL of acetone and 1.35 mL of H_2O , and the mixture was heated at reflux for 2 h and then stirred overnight at room temperature. The mixture was poured into H_2O and extracted with petroleum ether. The organic layer was washed successively with 2 N HCl, H_2O , 1 N $NaHCO_3$, and H_2O and dried over Na_2SO_4 . The crude product was chromatographed on silica gel to give 4-(trimethylgermyl)acetophenone (29, 97%), which was condensed with methyl terephthalaldehyde in *i*PrOH according to the method described for 21 to give 30. 30: pale-yellow needles (from CH_3OH); mp 230–231 °C; 1H NMR (400 MHz, $CDCl_3$) δ 0.44 (s, 9 H), 7.62 (d, 1 H, $J = 16$ Hz), 7.64 (d, 2 H, $J = 8$ Hz), 7.73 (d, 2 H, $J = 8$ Hz), 7.83 (d, 1 H, $J = 16$ Hz), 7.98 (d, 2 H, $J = 8$ Hz), 8.14 (d, 2 H, $J = 8$ Hz). Anal. ($C_{19}H_{20}O_3Ge$) C, H.

(E)-4-[3-[3,5-Bis(trimethylgermyl)phenyl]-3-oxo-1-propenyl]benzoic Acid (35, Ch55G). A mixture of 3,5-dibromoacetophenone³¹ (31, 824 mg, 2.96 mmol), ethylene glycol (279 mg, 4.49 mmol), and *p*-TsOH· H_2O (29 mg, 0.15 mmol) in 100 mL of dry benzene was heated at reflux for 34 h in a flask equipped with a Dean-Stark apparatus. The mixture was washed with 1 N K_2CO_3 and H_2O and dried over Na_2SO_4 . 2-(3,5-Dibromophenyl)-2-methyl-1,3-dioxolane (32) was obtained as colorless needles (76%, mp 74–74.5 °C). A solution of 32 (644 mg, 2.00 mmol) in 10 mL of THF was added to a mixture of Mg (108 mg, 4.44 mmol) and chlorotrimethylgermane (765 mg, 5.00 mmol) at 40 °C under Ar. The mixture was heated at reflux for 4.5 h and then stirred overnight at room temperature. The mixture was poured into ice-water and extracted with CH_2Cl_2 . The organic layer was washed with H_2O and dried over Na_2SO_4 . The crude product was chromatographed on silica gel to give 2-[3,5-bis(trimethylgermyl)phenyl]-2-methyl-1,3-dioxolane (33, 70%). Compound 33 (240 mg, 0.60 mmol) and pyridinium tosylate (23 mg, 0.09 mmol) were dissolved in 10 mL of acetone and 0.54 mL of H_2O , and the mixture was heated at reflux for 2.5 h. The solvent was removed and the residue was diluted with ether. The organic layer was washed successively with H_2O , 1 N $NaHCO_3$, and H_2O and dried over Na_2SO_4 . After the removal of the solvent, the crude product was purified by sublimation (0.5 mmHg, 70 °C) to give 3,5-bis(trimethylgermyl)acetophenone³² (34, 89%) as colorless prisms (mp 56 °C). Compound 34 was condensed with methyl terephthalaldehyde in THF-*i*PrOH according to the method described for 21 to give 35 (54%). 35: yellow prisms (from CH_2Cl_2 - CH_3OH -*n*-hexane), mp 195.5 °C dec; 1H NMR (400 MHz, $CDCl_3$) δ 0.45 (s, 18 H), 7.59 (d, 1 H, $J = 16$ Hz), 7.74 (d, 2 H, $J = 8$ Hz), 7.78 (t, 1 H, $J = 1$ Hz), 7.81 (d, 1 H, $J = 16$ Hz), 8.03 (d, 2 H, $J = 1$ Hz), 8.16 (d, 2 H, $J = 8$ Hz). Anal. ($C_{22}H_{26}O_3Ge_2$) C, H.

(E)-4-[3-Oxo-3-[3-(trimethylgermyl)phenyl]-1-propenyl]benzoic Acid (40, Ch60G). A mixture of 3-bromoacetophenone (36, 1.99 g, 10.0 mmol), ethylene glycol (931 mg, 15.0 mmol), and *p*-TsOH· H_2O (95 mg, 0.5 mmol) in 200 mL of dry benzene was heated at reflux in a flask equipped with a Dean-Stark apparatus for 4 days. After cooling, the mixture was washed with H_2O and dried over Na_2SO_4 . The crude mixture was chromatographed on silica gel to give 2-(3-bromophenyl)-2-methyl-1,3-dioxolane (37, 83%). A solution of 37 (729 mg, 3.00 mmol) in 10 mL of THF was added to a mixture of Mg turnings (81 mg, 3.33 mmol) and chlorotrimethylgermane (574 mg, 3.75 mmol) at 40 °C under Ar. The mixture was heated at reflux for 4.5 h and then stirred overnight at room temperature. The mixture was diluted with petroleum ether and filtered. The filtrate was evaporated and the residue was chromatographed on silica gel to give 2-methyl-2-[3-(trimethylgermyl)phenyl]-1,3-dioxolane (38, 78%) as colorless cubes. Compound 38 (351 mg, 1.25 mmol) and pyridinium tosylate (47 mg, 0.19 mmol) were dissolved in 10 mL of acetone and 1.13 mL of H_2O , and the mixture was heated at reflux for 2.5 h. The solvent was removed and the residue was

(30) Sterzycki, R. *Synthesis* 1979, 724.

(31) Fuchs, W. *Monatsh. Chem.* 1915, 36, 113.

(32) Wrobel, D.; Wannagat, U. *J. Organomet. Chem.* 1982, 225, 203.

diluted with petroleum ether. The organic layer was washed successively with H₂O, 1 N NaHCO₃, and H₂O and dried over Na₂SO₄. The crude product was chromatographed on silica gel to give 3-(trimethylgermyl)acetophenone (**39**, 99%), which was condensed with methyl terephthalaldehyde in THF-*i*PrOH according to the method described for **21** to give **40** (22%). **40**: pale-yellow prisms (from AcOEt-*n*-hexane); mp 186-188 °C; ¹H NMR (400 MHz, CDCl₃) δ 0.45 (s, 9 H), 7.50 (dd, 1 H, *J* = 7, 8 Hz), 7.62 (d, 1 H, *J* = 16 Hz), 7.72 (dt, 1 H, *J* = 1, 7 Hz), 7.74 (d, 2 H, *J* = 8 Hz), 7.83 (d, 1 H, *J* = 16 Hz), 7.96 (ddd, 1 H, *J* = 1, 2, 8 Hz), 8.11 (m, 1 H), 8.14 (d, 2 H, *J* = 8 Hz). Anal. (C₁₈H₂₀O₃Si¹/₄H₂O) C, H.

(*E*)-4-[2-[3-(Trimethylsilyl)phenyl]ethenyl]benzoic Acid (**45**, **St40S**). A solution of *m*-bromotoluene (**41**, 7.7 g, 45 mmol) in 20 mL of dry ether was added slowly to 30% lithium dispersion in oil (2.32 g, 0.10 mol) in 10 mL of dry ether at -10 °C under Ar and the mixture was stirred for 3 h at room temperature. A solution of chlorotrimethylsilane (4.68 g, 43.1 mmol; freshly distilled from CaH₂) in 14 mL of dry ether was added, and the mixture was stirred for 36 h. After filtration, the filtrate was washed with H₂O and brine and dried over Na₂SO₄. The crude mixture was chromatographed on silica gel to give *m*-tolyltrimethylsilane (**42**, 54%). *N*-Bromosuccinimide (1.09 g, 6.12 mmol) and a catalytic amount of azobisisobutyronitrile were added to a solution of **42** (1.0 g, 6.09 mmol) in 6 mL of dry CH₂Cl₂ and the mixture was refluxed for 4.5 h. After cooling and filtration, the solvent was removed. The residue was chromatographed on silica gel to give 3-(trimethylsilyl)benzyl bromide (**43**, 70%). A mixture of **43** (730 mg, 3.00 mmol) and triphenylphosphine (1.18 g, 4.50 mmol) was refluxed in 10 mL of dry toluene under Ar for 4 h. [3-(Trimethylsilyl)benzyl]triphenylphosphonium bromide (**44**) was obtained by filtration (90%). Compound **44** (758 mg, 1.50 mmol) and methyl terephthalaldehyde (258 mg, 1.57 mmol) were added to a solution of Na (40 mg, 1.74 mmol) in 15 mL of dry

methanol, and the mixture was stirred for 17 h. The precipitate was collected to give methyl (*E*)-4-[2-[3-(trimethylsilyl)phenyl]ethenyl]benzoate (**St41S**). The filtrate was chromatographed on silica gel to give **St41S** (total 70%) and its *Z* isomer (24%). **St41S** was hydrolyzed (aqueous KOH-EtOH) to give **45** (**St40S**, 85%). **St41S**: colorless needles (from CH₂Cl₂-*n*-hexane); mp 113-114.5 °C; ¹H NMR (100 MHz, CDCl₃) δ 0.30 (s, 9 H), 3.92 (s, 3 H), 7.0-7.6 (m, 8 H), 8.02 (d, 2 H, *J* = 8 Hz); UV λ_{max} (nm, log ε) 323 (4.91), 232 (4.61), 209 (4.75). **45** (**St40S**): colorless needles (from CH₂Cl₂-*n*-hexane); mp 216-218 °C; ¹H NMR (100 MHz, CDCl₃-DMSO-*d*₆) δ 0.30 (s, 9 H), 7.12 (d, 1 H, *J* = 18 Hz), 7.25 (d, 1 H, *J* = 18 Hz), 7.4-7.8 (m, 6 H), 7.95 (d, 2 H, *J* = 8 Hz). Anal. (C₁₈H₂₀O₂Si¹/₄H₂O) C, H.

Registry No. 3, 541-73-1; 4, 2060-89-1; 5, 15290-24-1; 6, 15290-25-2; 7, 125973-48-0; 8, 626-39-1; 9, 5624-60-2; 10, 78627-91-5; 11, 125973-49-1; 12, 125973-50-4; 13, 125973-51-5; 14, 125973-52-6; 15, 125973-53-7; 16, 125973-54-8; 17, 81500-98-3; 18, 125973-55-9; 19, 125973-56-0; 20, 17983-62-9; 21, 125973-57-1; 22, 125973-58-2; 23, 99-90-1; 24, 4360-68-3; 25, 18192-28-4; 26, 17983-61-8; 27, 125973-59-3; 28, 81805-70-1; 29, 81805-71-2; 30, 125973-60-6; 31, 14401-73-1; 32, 81500-93-8; 33, 81500-96-1; 34, 81500-99-4; 35, 125973-61-7; 36, 2142-63-4; 37, 39172-32-2; 38, 81805-70-1; 39, 125973-62-8; 40, 125973-63-9; 41, 591-17-3; 42, 3728-44-7; 43, 17903-44-5; 44, 125973-64-0; 45, 125973-65-1; 46, 102121-16-4; 47, 116232-90-7; 48, 104182-40-3; 49, 119567-94-1; 50, 110368-33-7; 51, 119567-96-3; 52, 102405-34-5; Am41S, 125973-66-2; Am56S, 125973-69-5; TMGCl, 1529-47-1; Am56G, 125973-70-8; Am556S, 125973-71-9; **St41S** (*E* isomer), 125973-72-0; **St41S** (*Z* isomer), 125973-73-1; *p*-ClCOC₆H₄COOCH₃, 7377-26-6; *p*-NH₂C₆H₄COOH₃, 619-45-4; *p*-HCOC₆H₄COOCH₃, 1571-08-0; 1-nitro-3,4-bis(trimethylsilyl)benzene, 78627-92-6; 1,3-dinitro-5-(trimethylsilyl)benzene, 125973-47-9; 3,3',5,5'-tetrakis(trimethylsilyl)biphenyl, 125973-67-3; 2-nitro-1,3,5-tris(trimethylsilyl)benzene, 125973-68-4.

Synthesis and Pharmacological Evaluation of Amino, Azido, and Nitrogen Mustard Analogues of 10-Substituted Cannabidiol and 11- or 12-Substituted Δ⁸-Tetrahydrocannabinol

David R. Compton,*† Patrick J. Little,† Billy R. Martin,† Jeffery W. Gilman, Jayanta K. Saha, Vaman S. Jorapur, Howard P. Sard,† and Raj K. Razdan†

Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298, and Sisa Pharmaceutical Laboratories, Inc., Cambridge, Massachusetts 02138. Received June 12, 1989

The synthesis of a variety of novel 10-substituted cannabidiol (CBD) and 11- or 12-substituted Δ⁸-tetrahydrocannabinol (Δ⁸-THC) analogues containing amino, alkylamino, azido, or a *N,N*-bis(2-chloroethyl)amino functional group is described, as well as their pharmacological evaluation in mice. These analogues, which possess only a portion of the full pharmacological spectrum of activity of Δ⁹-THC, indicate that cannabinoid-mediated reduction of spontaneous locomotor activity, hypothermia, antinociception, and/or catalepsy need not be produced simultaneously, possibly suggesting the existence of more than one mechanism of action. The 10-substituted CBD analogues **3**, **4**, and **5** with an ethylamino, propylamino, or azido functional group, respectively, proved to be largely inactive, except for the production of central nervous system (CNS) depression concomitant with toxicity. Toxicity and CNS depression may be related phenomena in these nitrogenous compounds since 12-amino and 12-ethylamino analogues (**8** and **11**) of Δ⁸-THC also proved to be very toxic. Antinociceptive and hypothermic responses (without reduction of motor activity) were observed at a dose of 10 mg/kg of the 11-ethylamino analogue (**9**) of Δ⁸-THC, while a dose of 50 mg/kg of the nitrogen mustard 11-[*N,N*-bis(2-chloroethyl)amino]-Δ⁸-THC (**12**) was necessary to produce any observable pharmacological effect. When selected analogues were evaluated for antagonistic properties, they failed to attenuate the effects of Δ⁹-THC. Some nitrogen mustard analogues were capable of producing minimal pharmacological effects after either peripheral or direct CNS administration; however, these analogues also failed to attenuate the effects of Δ⁹-THC either immediately after administration or 24-48 h later.

Δ⁹-Tetrahydrocannabinol (Δ⁹-THC) and certain analogues (e.g. Δ⁸-THC) produce characteristic psychotropic responses in humans, as well as specific behavioral alter-

ations in laboratory animals.¹ Other naturally occurring cannabinoids, such as cannabidiol (CBD, **1**), have been found to be devoid of many of these pharmacological effects, as well as devoid of psychoactive properties.² The pharmacological effects of the cannabinoids have been

* Author to whom inquiries should be addressed.

† Virginia Commonwealth University.

‡ Present address: Organix, Inc., 65 Cummings Park, Woburn, MA 01801.

(1) Dewey, W. L. *Pharmacol. Rev.* **1986**, *38*, 151.

(2) Razdan, R. K. *Pharmacol. Rev.* **1986**, *38*, 75.