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A NEW ANTINEOPLASTIC METHYLGERMANIUM(IV)PORPHYRIN

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A new highly lipophilic methylgermanium(IV)porphyrin; dimethyl-5,10,15,20-tetrakis[3',5'-bis(1",1"-dimethylethyl)phenyl]porphynato-germanium(IV) (Abbr. MGP), has been prepared and characterized. The isolated compound showed considerable activity toward neoplastic tissues both *in vitro* and *in vivo*.

Ever since the discovery of the remarkable antitumor activity of *cis*-dichlorodiammineplatinum(II) (cisplatin), much efforts have been spent for the amerioration of the original complex together with the biochemical and biophysical studies of cisplatin.[1] Little work, however, has been carried out toward the development of a new class of inorganic anticancer agents using another metals.[2-4] In contrast to the well-developed area of organic anticancer agents,[5] an ample amount of attractive nature in inorganic compounds is left largely untouched for designing of a novel type of antineoplastic reagents. These include such well known properties as i) the tendency of tetraphenylporphyrin derivatives to accumulate in malignant tissues,[6] ii) potential utility of metal alkyls to act as an alkylating agent, iii) the nature of elemental germanium to suppress mouse spontaneous tumors.[7] It is therefore feasible to assume an outcome of a new class of inorganic anticancer agents combining the nature mentioned above.

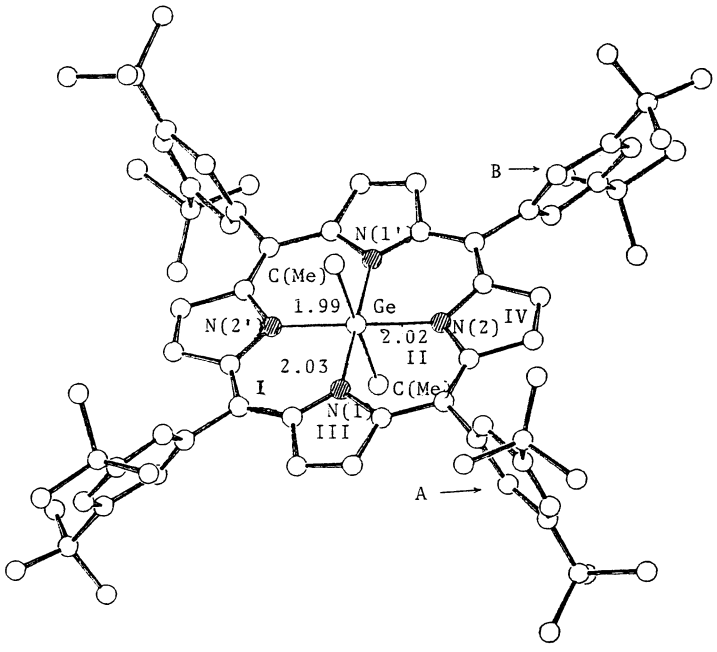

Fig. 1. Perspective drawing of the complex and selected bond distances in Å.[11]

We wish to report here characterization and *in vitro* tests of a new highly lipophilic ger-

manium porphyrin; dimethyl-5,10,15,20-tetrakis[3',5'-bis(1",1"-dimethylethyl)-phenyl]porphinatogermanium(IV) (Abbr. MGP).

MGP was prepared by the literature methods.[8] The obtained complex is stable toward air, but it gradually decomposes under visible light. Especially, decomposition rapidly proceeds in solution.[9]

The blue-green plate crystals wich were submitted to the X-ray structure analysis,[10] were obtained from the benzene solution. Figure 1 shows a perspective drawing of the complex. Ge lies on a center of symmetry of the unit cell and the two methyl groups are directly bonded to Ge at the both faces of molecular plane [Ge-C(Me), 1.99(3) Å]. The bond distances of Ge-N (in pyrrole ring), 2.02(4) and 2.03(4), are in agreement with the predicted value of 1.98 Å.[12] The angle formed by the Ge-C(Me) bond and the plane defined by four porphyrin nitrogen atoms and Ge makes 86.9°. Dihedral angles between the phenyl rings and the plane made by porphyrin skeleton are not unique. The phenyl group (A) makes 85.1°, almost perpendicular to the porphyrin ring, while the other one (B) is tilted to form 64.0° with the porphyrin skeleton.

In addition to the above crystallographic evidence, the presence of methyl-Ge direct bond was confirmed again in a solution by the fact that both pyrrole β-H and methyl protons in ^{1}H-NMR appeared as singlet peaks.

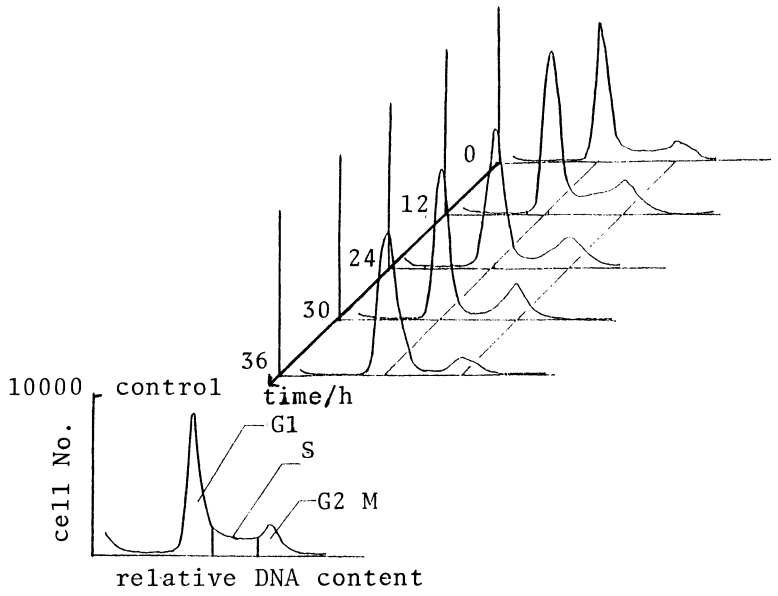

Fig. 2. Obtained DNA histogram. Instrument used: FACS-IIIμ Becton Dickinson, CA., U.S.A. 488 nm laser beam.

MGP showed expected activity toward HeLa cells *in vitro*. Remarkable cytotoxic effects were observed at the concentration of 15 μg/ml and the total necrosis of the cells seeded onto culture dish was confirmed at the concentration of 20 μg/ml in the colony formation assay. The cell cycle dependency of MGP drug action was also studied using flow cytometry.[13] After the dose(20 μg/ml), cells were collected every 12 h and relative DNA content was measured. Obtained time-dependent DNA histograms are shown in Fig. 2 and the change of relative cell content (in %) in each phase calculated by integration of an each histogram is shown in Fig. 3. These two figures clearly indicate the marked decrease in the relative content of S-phase after 12 h. In other words, MGP is an S-phase blocking agent.

The compound also exhibited antineoplastic activity against three types of solid tumors *in vivo*. Three long-term survivors with the complete remission of

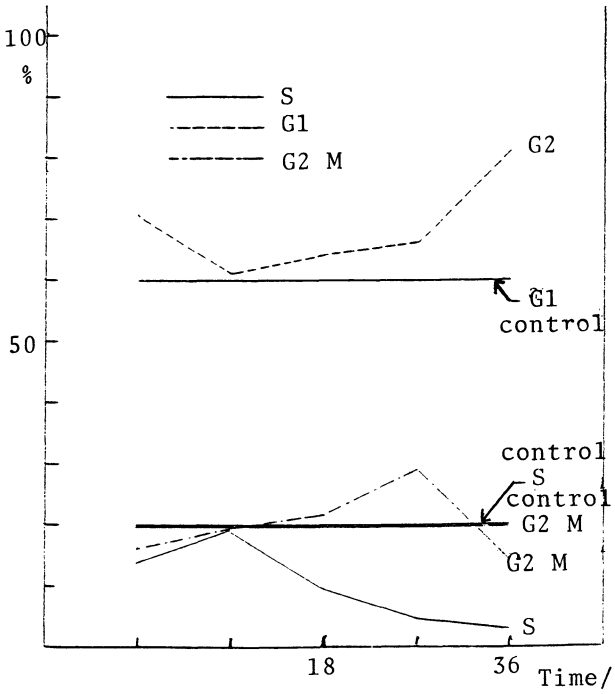

Fig. 3. The change of relative cell content in each phase.

Walker 256 carcinosarcoma were observed out of six Donryu rats at the dose of 100 mg/kg. Best T/C[14] 61% at the dose of 80 mg/kg was obtained for IMC carcinoma and 67% for B-16 melanoma.

Considering the fact that the corresponding dichlorogermanium(IV)-porphyrin, *i.e.*, dichloro-5,10,15,20-tetrakis[3',5'-bis(1",1"-dimethylethyl)phenyl]porphinatogermanium(IV), is ineffective in animal antitumor tests, activation of the methyl groups attached to Ge might play a significant role in the anticancer drug action.

Syntheses of analogous alkylmetal complexes are now in progress.

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7) M. Kanisawa and H. A. Schroeder, Cancer Res., 27, 1192 (1967).
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1.5 (72H, s, t-butyl H), -2.6 (2H, s, internal pyrrole H). UV-Vis/nm in chloroform ($\varepsilon \times 10^{-3}$): 421(561), 515(21), 551(11). Solubility of free base is extremely enhanced in pentane (25°C) by the factor of 390 compared to tetraphenylporphyrin. The same argument is extended to the metal complexes of Co(II), Ni(II), Cu(II), and Zn(II). The analytically pure MGP was obtained by the use of Merck alumina 90 column chromatography. Found: C, 80.42; H, 8.31; N, 4.75%. Calcd for $C_{79}H_{98}N_4Ge$: C, 80.47; H, 8.48; N, 4.81%. ^{1}H-NMR in $CDCl_3$: δ 8.9 (8H, s, β-pyrrole H), 8.1 (8H, d, ortho phenyl H), 7.8 (4H, t, para phenyl H), 1.5(72H, s, t-butyl H), -6.9 (6H, s, methyl H). UV-Vis/nm in benzene: 450, 593, 638.

9) The decomposition was confirmed by the disappearance of the original signals of the methyl, t-butyl, phenyl and β-pyrrole protons in ^{1}H-NMR spectrum.

10) We were fortunate to obtain single crystals of MGP, though these turned out to be only marginally suitable for X-ray diffraction studies. In spite of poor crystal quality and limited data, we have been able to determine the main structural features of MGP. Until more precise structure become available, it seems foolish to ascribe any significance to the subtle differences of the bond angles and distances. All operations were carried out under dark or dim light due to the photosensitivity of MGP. Obtained crystal data: $C_{78}H_{98}N_4Ge \cdot 4C_6H_6$, Mr = 1576.72; monoclinic, $P2_1/c$; a = 15.392(3) Å, b = 17.237(3) Å, c = 18.492(3) Å; β = 115.88(3)°; V = 4399.2(1.1) Å^3; Z = 2, D_x = 1.115 g/cm^3, μ(Mo Kα) = 4.2 cm^{-1}; crystal dimensions, 0.28 x 0.25 x 0.065 mm^3. The crystal was sealed into a thin-walled capillary tube. Intensity data were collected on a Rigaku automated four-circle diffractometer, using Mo Kα radiation monochromated by a graphite plate. Of the 4173 total reflections collected for which $2\theta \leq 40°$, independent 1233 with $|Fo| \geq 3\sigma(Fo)$ were used for the refinement. The intensity data were normalized to interpolated value of the net count of the four standard reflections. The structure was solved by the heavy-atom method and refined by the least-squares method to R = 0.147 by using anisotropic thermal parameters for Ge atom and isotropic ones for all the other non-hydrogen atoms.

11) Primed and unprimed atoms are related by crystallographic symmetry. The bond angles (degrees) are: N(1)-Ge-N(2), 90.5(1.5); N(2)-Ge-N(1'), 89.6(1.5); N(1)-Ge-C(Me), 91.7(1.4); N(2)-Ge-C(Me), 92.6(1.3). The departure from planarity of the porphyrin ring is estimated in terms of the following dihedral angles (degrees) between the least-squares planes of six-membered rings (I and II) and five-membered rings (III and IV): I-III, 3.4; I-IV, 5.9; II-III, 2.5; II-IV, 4.1.

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14) T/C % = tumor weight (volume) of treated animals/tumor weight (volume) of controlled animals x 100, T/C % < 42, +; 42 < T/C % < 62, ±; T/C % > 65, -.

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