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SYNTHESES OF 5-FLUOROURACIL-TERMINATED MONOMETHOXYPOLY(ETHYLENE GLYCOL)S, THEIR HYDROLYSIS BEHAVIOR, AND THEIR ANTITUMOR ACTIVITIES

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ABSTRACT

The covalent attachments of 5-fluorouracil (5FU) units to poly(ethylene glycol) monomethoxy ether (MeO-PEG) attached through ester, amide, and ether bonds were carried out; three types of linkages were obtained through which 5FU units were attached to MeO-PEG. For the investigation of the release of the 5FU units, the homogeneous hydrolysis was investigated *in vitro* in the presence and absence of enzymes. Although the rate of release of 1-β-carboxyethyl-5FU or 1-β-hydroxyethyl-5FU from the compounds was fast, the release of 5FU itself was slow. In addition, the antitumor activity of these three types of MeO-PEG-bound 5FU unit was tested *in vivo* by preliminary screening by the National Cancer Institute or by the Japanese Foundation for Cancer Research.

INTRODUCTION

5-Fluorouracil (5FU) has not only a remarkable antitumor activity, but this activity is also accompanied by strong side effects [1, 2]. In order to eliminate these side effects, 5FU had been converted to 1-(tetrahydro-2-furanyl)-5-fluorouracil [3] and 5-fluorouridine [4] compounds for use in practice.

A polymeric drug such as 5FU attached to a polymer can be expected to have reduced toxicity, increased duration of antitumor activity, and, additionally, a possible affinity for tumor cells.

In previous papers we reported on the synthesis of polyacrylates of 3-(5-fluorouracil-1-yl)propanoic acid (1-β-CE-5FU (**3**)) which were attached through D-glucofuranose groups via ester bonds [5]. The synthesis of polyether-bound **3** via pendant ester bonds (pendant-ester type poly-ether/5 FU unit) [6] was also accomplished; at the time we commented on the antitumor activities of these compounds.

In our continued efforts to attach biologically active compounds to polymers and oligomers, we selected poly(ethylene glycol) (PEG) as the carrier of biologically active compounds. PEG is compatible with living system, it is a water-soluble macromolecular carrier, and it is reported to be of low toxicity. In a preceding paper [7] we reported briefly on the covalent attachment of **3** to monomethoxy-poly(ethylene glycol) (MeO-PEG; **6**; the number of ethylene oxide (EO) units, *n*, 1-110) through an ester bond, and on the hydrolysis of the resulting compound in the presence or absence of enzymes *in vitro*. We also reported on the antitumor activity of **6** obtained by attaching **3** onto MeO-PEG through an ester bond (α-[3-(5-fluorouracil-1-yl)propionoyl]-ω-poly-(ethylene glycol) methyl ether) (MeO-PEG/ester/1-β-CE-5FU) (**7**), which has a polyether/5FU unit attached to the end of the PEG chain. We then found that the rate of hydrolysis of the ester bond decreased and the antitumor activity increased with an increasing number *n* of EO units in MeO-PEG. We also investigated the effect of the number of EO units in MeO-PEG and the types of bonds between MeO-PEG and the 5FU units on the efficiency of the hydrolysis of the ester bond and the antitumor activity of the PEG having a 5FU unit attached at the end of the chain.

The present paper deals with the type of covalent attachments of 5FU units to MeO-PEG, especially the attachment through ester, amide, or ester bonds, using MeO-PEG of differing number of groups as the base oligomers. We synthesized specifically: (a) MeO-PEG/ester/1-β-CE-5FU (**7**) with *n* of 1 to 230 and **3** attached through amide bonds (α-[3-(5-fluorouracil-1-yl)propionyl]-amino-ω-poly(ethylene glycol) methyl ether); (b) MeO-PEG/amide/1-β-CE-5FU

(**11**) with n between 1 and 110 and **3** attached to MeO-PEG through an ether bond (α-(5-fluorouracil)-ω-poly(ethylene glycol) methyl ether); (c) MeO-PEG/ether/1-β-HE-5FU (**14**) with n of 1 to 110. The hydrolyses of the end ester, amide, and ether bonds in **7**, **11**, and **14** in the presence or absence of enzymes were investigated *in vitro*. In addition, the antitumor activities of **7**, **11**, and **14** were evaluated by preliminary screening test at the National Cancer Institute (NCI) or at the Japanese Foundation for Cancer Research (JFCR).

EXPERIMENTAL

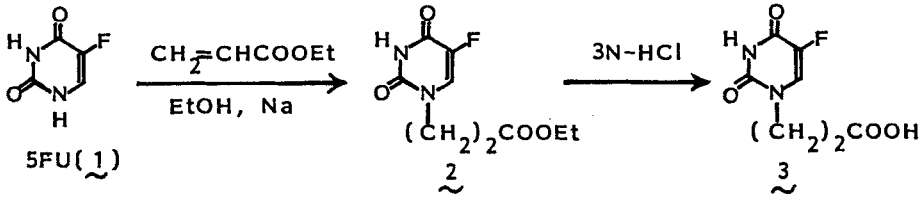
Tetrahydrofuran (THF), 1,4-dioxane (DO), methanol. ethanol, pyridine, benzene, chlorobenzene, and ethyl acrylate were purified as usual. 5FU, sodium, 3 *N* HCl, cyanuric chloride (**4**), triethylamine (TEA), potassium *t*-butoxide, bromoacetaldehydediethylacetal, potassium hydroxide, sodium hydroxide, sodium cyanoborohydride, dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide (HONSu) dibromosulfoxide, and tetra-*n*-butylammonium bromide were commercial grade reagents and used without further purification.

MeO-PEG samples of n = 1 or 4 (**6a, 6b**) were distilled twice and MeO-PEG of n = 7, 15, 110, and 230 (**6c, 6d, 6e, 6f**) were freeze-dried from benzene before use.

Compound **3**, as an example of a 5FU unit, was prepared from 5FU and ethyl acrylate by the method of Rempp [8] : mp 194-195°C (Scheme 1).

Synthesis of MeO-PEG/Ester/1-β-CE-5FU (7)

Without isolation of 3-(5-fluorouracil-1-yl)propionyl chloride (**5**) (obtained from **3** by the cyanuric acid chloride (**4**)) [9],



SCHEME 1.

dry MeO-PEG (**6**) was allowed to react with **5** to give **7**. TEA (0.02 mol) was added to a solution of **3** (0.02 mol) and **4** (0.01 mol) in dry THF (20 mL) at room temperature. After the reaction was complete, a solution of **6** (0.02 mol) and TEA (0.02 mol) in THF was added dropwise to the reaction mixture, which was stirred at room temperature for 2 h and then heated to reflux for 2 h (**7a**), 4 h (**7b**), 6 h (**7c**), 15 h (**7d**), 48 h (**7e**), or 48 h (**7f**). (The progress of each reaction was monitored by TLC on Merke F_{254} silica gel plates). After the reaction was complete, the TEA salt of hydrochloride was removed by filtration and the filtrate evaporated under reduced pressure. Crude **7** was obtained, which was subjected to medium-pressure column chromatography on Kieselgel 60. The eluent obtained by varying the developing solvents from ethyl acetate/chloroform (5:4 v/v) to ethyl acetate/chloroform/methanol (10:7:1 v/v) gave the product **7** as needles, viscous oil, or powder. **7a-7f** were similarly obtained and identified by their IR, ^{1}H-NMR, and UV spectra as well as TLC and HPLC analyses.

IR (neat or KBr pellet) showed absorptions at 3200 (NH), 3100-2850 (CH), 1735 (C=O), 1690 (amide C=O) and 1485-1420 cm^{-1} (CH_2).

^{1}H NMR (D_2O) showed δ2.61-3.10 (t, 5FU-C$\underline{H}_2$C$\underline{H}_2$), 3.24-3.64 (m, C$\underline{H}_3$OC$\underline{H}_2$C$\underline{H}_2$), 3.86-4.21 (t, 5FU-CH$_2$C$\underline{H}_2$), 7.84-7.94 (s, H-6 of 5FU) and 10.21-10.80 ppm (broad, NH).

TLC monitored with UV_{254} and I_2 (developing solvent: *n*-butanol/ethanol/water of 4:1:1 in v/v) gave R_f = 0.80 (**7a**), 0.79 (**7b**), 0.78 (**7c**), 0.76 (**7d**), 0.67 (**7e**), and 0.65 (**7f**).

Synthesis of MeO-PEG/Amide/1-β-CE-5FU (11)

MeO-PEG-aldehyde (**9**) and MeO-PEG-amine (**10**) were prepared by the method of Harris [10].

MeO-PEG-Aldehyde (**9**). Potassium *t*-butoxide (0.04 mol) was added to 0.04 mol of **6** in 30 mL of dry benzene, and the mixture was stirred at room temperature for 30 min. Bromoacetaldehydediethylacetal (0.04 mol) in dry benzene (5 mL) was added dropwise and stirred for 12 h. The reaction mixture was added to dry ethyl ether (200 mL), and the resulting MeO-PEG-acetal was filtered, placed in 20 mL of 2 *N* HCl, and stirred for 30 min. Ethanol (50 mL) was added, and the solution was held at 0°C for 24 h; crystalline α-formyl-ω-methoxypoly(oxyethylene) (MeO-PEG-aldehyde; **9**) was obtained.

MeO-PEG-Amine (**10**). Sodium hydroxide (0.4 mmol) was added to ammonium chloride (0.7 mmol) in 5 mL methanol. After the NaOH pellets had dis-

solved, **9** (0.67 mmol) was added and the solution stirred for 30 min. A solution of sodium cyanoborohydride (6.7 mmol) in 5 mL methanol was then added dropwise over 30 min, followed by the addition of sodium hydroxide (1.8 mmol). The solution was stirred for 12 h (n = 1), 16 h (n = 4), 18 h (n = 15), or 24 h (n = 110), and the product was then precipitated by the addition of 200 mL dry ethyl ether. Gummy α-amino-ω-methoxypoly(oxyethylene) (MeO-PEG-amine; **10**) was isolated by centrifugation.

MeO-PEG/Amide/1-β-CE-5FU (**11**). Compound **11** was synthesized by coupling **3** with **10** using the active ester method. DCC (1 mmol) and HONSu (1.2 mmol) were added to the ice-cold solution of **3** (1 mmol) in dry pyridine (30 mL). The reaction mixture was stirred at 4°C for 1 h, and *N,N'*-dicyclohexylurea which had formed was removed by filtration and washed with pyridine. **10** (1 mmol) and TEA (1 mmol) were added to the combined filtrate and the washings cooled in an ice bath. The mixture was stirred at room temperature for 30 min and heated to reflex for 10 h (n = 1), 12 h (n = 4), 18 h (n = 15), or 24 h (n = 110). The progress of each reaction was monitored by TLC on Merck Art 9385 Kieselgel 60 plates. The reaction mixture was concentrated by evaporation, extracted with ethyl acetate, and the solution was decanted from a small amount of an insoluble portion. Evaporation of the ethyl acetate layer gave a syrup which was freeze-dried from water (crude product **11**). The eluent obtained by varying the developing solvents from ethyl acetate/chloroform (4:3 v/v) to ethyl acetate/chloroform/methanol (5:4:1 v/v) gave product **11**, which was freeze-dried. **11a-11d** were identified by their IR, ^{1}H-NMR, and UV spectra, and TLC and HPLC analyses.

IR (neat NaCl) showed absorption bands at 3200 (NH), 3100-2870 (CH), 1690-1730 (amide CONH) and 1485-1420 cm^{-1} (CH_2).

^{1}H NMR (D_2O) showed δ1.80-3.14 (t, 5FU-C$\underline{H}_2$CH_2), 3.24-3.54 (m, C$\underline{H}_3$OC$\underline{H}_2$C$\underline{H}_2$), 3.86-4.18 (t, 5FU-CH$_2$C$\underline{H}_2$), 6.80-7.80 (broad, CO$\underline{N}$H), 7.62-7.89 (s, H-6 of 5FU), and 10.12-10.75 ppm (broad, NH).

TLC was monitored with UV_{254} and I_2 (developing solvent: n-butanol/ethanol/water of 4:1:1 in v/v): R_f = 0.78 (**11a**), 0.78 (**11b**), 0.76 (**11c**), and 0.64 (**11d**).

Synthesis of MeO-PEG/Ether/1-β-HE-5FU (14)

Compound **14** was prepared by the coupling reaction of the potassium salt of 5-fluorouracil (5FU) (**12** with α-bromo-ω-methoxypoly(ethylene oxide) (MeO-PEG-bromide; **13**)).

Potassium Salt of 5FU (K5FU; **12**). **12** was prepared by the method of Ozaki [11]. Potassium hydroxide (4.0 mmol) was added to 5FU (4.0 mmol) in 20 mL dry methanol, and the mixture was stirred at room temperature for 1 h. Methanol and water which had formed were evaporated under reduced pressure to give K5FU (**12**).

MeO-PEG-Bromide (**13**). **13** was prepared by the method of Buckmann [12]. Dry MeO-PEG (**6**; 4.0 mmol) and TEA (4.0 mmol) were dissolved in 50 mL dry benzene. Within 1 h, 4.5 mmol freshly distilled thionyl bromide was added dropwise at 35°C with continuous stirring under a dry nitrogen atmosphere. After heating to reflux for 1 h (n = 1), 4 h (n = 4), 16 h (n = 15), or 24 h (n = 110), the reaction mixture was held at room temperature for 4 h. Triethylammonium bromide, which had precipitated, was filtered off; benzene and unreacted thionyl bromide were removed under reduced pressure; pale yellow **13** was obtained as an oil or powder.

IR (neat NaCl) showed a typical absorption at 700 cm^{-1} for a C–Br bond.

MeO-PEG/Ether/1-β-HE-5FU (**14**). **12** (4.0 mmol), **13** (4.0 mmol), and tetra-*n*-butylammonium bromide (6.0 mmol) as a phase-transfer catalyst were added to 100 mL chlorobenzene. The mixture was heated to reflux for 10 h (n = 1), 12 h (n = 4), 24 h (n = 15), or 50 h (n = 110); the progress of each reaction was monitored by TLC on Merck Art 9385 Kieselgel 60 plates.

After removing the precipitated tetra-*n*-butylammonium bromide by filtration, the filtrate was concentrated and the residue obtained was poured into water. The precipitated product was freeze-dried (crude product), and the residue was purified from free bromide, **12**, and other salts by column chromatography on cation- and anion-exchange resins. The main fraction was subjected to medium-pressure column chromatography on Kieselgel 60. The eluate obtained by varying the developing solvents from ethyl acetate/chloroform (5:4 v/v) to ethyl acetate/chloroform/methanol (10:7:3 v/v) gave the desired **14**, which was freeze-dried. **14a-14d** were identified by their IR, ^{1}H-NMR, and UV spectra, and TLC and HPLC analyses.

IR (neat NaCl) showed absorption bands at 3200 (NH), 2850-2825 (CH), 1485-1420 (CH_2), and 1150 cm^{-1} (C–O).

^{1}H NMR (D_2O) showed δ 1.30-2.24 (t, 5FU-C$\underline{H}_2$C$\underline{H}_2$), 3.35-3.44 (m, C$\underline{H}_3$OC$\underline{H}_2$C$\underline{H}_2$), 3.61-3.80 (t, 5FU-CH$_2$C$\underline{H}_2$), 7.62-7.91 (broad, 6-H of 5FU), and 10.20-10.50 ppm (broad, NH).

TLC was monitored by UV_{254} and I_2 (developing solvent: *n*-butanol/water of 4:1:1 in v/v): R_f = 0.81 (**14a**), 0.80 (**14b**), 0.78 (**14c**), and 0.69 (**14d**).

Determination of the Extent of Hydrolysis of the Esters, Amides, and Ethers

The hydrolysis of the compound with MeO-PEG-bound 5FU units which were attached through ester, amide, and ether bonds was carried out *in vitro* at 37°C under shaking in systems without enzymes: 0.01 *N* NaOH, 0.1 *N* HCl, pseudohumor (aqueous solutions containing 0.6% w/v sodium chloride, 0.02% w/v calcium chloride, 0.03% w/v potassium chloride, 0.31% w/v sodium lactate, and 5.00% w/v dextrose), physiological aqueous saline solutions, and 1/15 *M* KH_2PO_4-Na_2HPO_4 buffer solution; or in systems with enzymes: esterase (Sigma Chemical Co., type I from porcine liver), α-chymotrypsin (P-L Biochemicals, Inc., from bovine pancreas), lipase (Sigma Chemical Co., type II from bovine pancreas), acrylase (Sigma Chemical Co., from orange peel), and α-amylase (Sigma Chemical Co., bacterial crude type III-A grade).

The extent of hydrolysis was determined by measuring the amount of 5FU that had been released, **3** or 1-β-hydroxyethyl-5FU (1-β-HE-5FU; **15**) using HPLC (column: Chemco Pak 05587, eluent: 1/75 *M* KH_2PO_4-Na_2HPO_4 buffer of pH 7.0, detector: UV_{271}) or GPC (column: Shodex OHpak B-803, eluent: 1/60 *M* KH_2PO_4-Na_2HPO_4 buffer solution of pH 7.0, detector: UV_{271}).

Measurement of Antitumor Activity

The antitumor activity was tested against p-388 *lymphocytic leukemia* in female CDF_1 mice *in vivo* (i.p./i.p.) according to methods typically used at NCI or JFCR. The test samples were sonicated in 0.05% Sorbate 80 in sterile normal saline solution and administered intraperitoneally. At the test start, 1×10^6 cancer cells were injected i.p. The mice received doses of 0-1600 mg/kg for 9 days (NCI) or at 1 and 5 days (JFCR). The ratio of prolongation of life, T/C, after 10 days, was determined.

RESULTS AND DISCUSSION

Synthesis of MeO-PEG Compounds with 5FU Units

The covalent attachments of **3** and **15** to MeO-PEG through ester, amide, and ether bonds were performed via the coupling reactions shown in Schemes 2-4 and gave the desired MeO-PEG/ester/1-β-CE-5FU (**7**), MeO-PEG/amide/1-β-CE-5FU (**11**), and MeO-PEG/ether/1-β-HE-5FU (**14**). The compounds were identified by their IR, ^{1}H-NMR, and UV spectra, and TLC and HPLC

TABLE 1. Yield and Characterization of MeO-PEG-Bound 5FU

No.	MeO-PEG(n)/bond/5FU[a]	Yield,[b] %	Form	mp, C°	λ_{max}, nm	ϵ_{max}, $L \cdot mol^{-1} \cdot cm^{-1}$
7a[c]	MeO-PEG(1)/ester/1-β-CE-5FU	58	Needles	56.0-56.5	270	9470
7b	MeO-PEG(4)/ester/1-β-CE-5FU	30	Viscous oil	–	271	9030
7c	MeO-PEG(7)/ester/1-β-CE-5FU	30	Viscous oil	–	270	9320
7d	MeO-PEG(15)/ester/1-β-CE-5FU	53	Waxy	–	270	9600
7e	MeO-PEG(110)/ester/1-β-CE-5FU	62	Powder	66.0-66.5	270	9000
7f	MeO-PEG(230)/ester/1-β-CE-5FU	60	Powder	69.2-70.0	270	9010
11a	MeO-PEG(1)/amide/1-β-CE-5FU	38	Needles	43.5-44.5	271	9610
11b	MeO-PEG(4)/amide/1-β-CE-5FU	30	Viscous oil	–	270	9030
11c	MeO-PEG(15)/amide/1-β-CE-5FU	24	Waxy	–	270	9460
11d	MeO-PEG(110)/amide/1-β-CE-5FU	47	Powder	67.5-68.0	269	9000
14a[d]	MeO-PEG(1)/ether/1-β-HE-5FU	60	Viscous oil	–	270	9310
14b	MeO-PEG(4)/ether/1-β-HE-5FU	47	Viscous oil	–	270	9020
14c	MeO-PEG(15)/ether/1-β-HE-5FU	58	Waxy	–	270	9030
14d	MeO-PEG(110)/ether/1-β-HE-5FU	67	Powder	70.0-70.5	270	9010

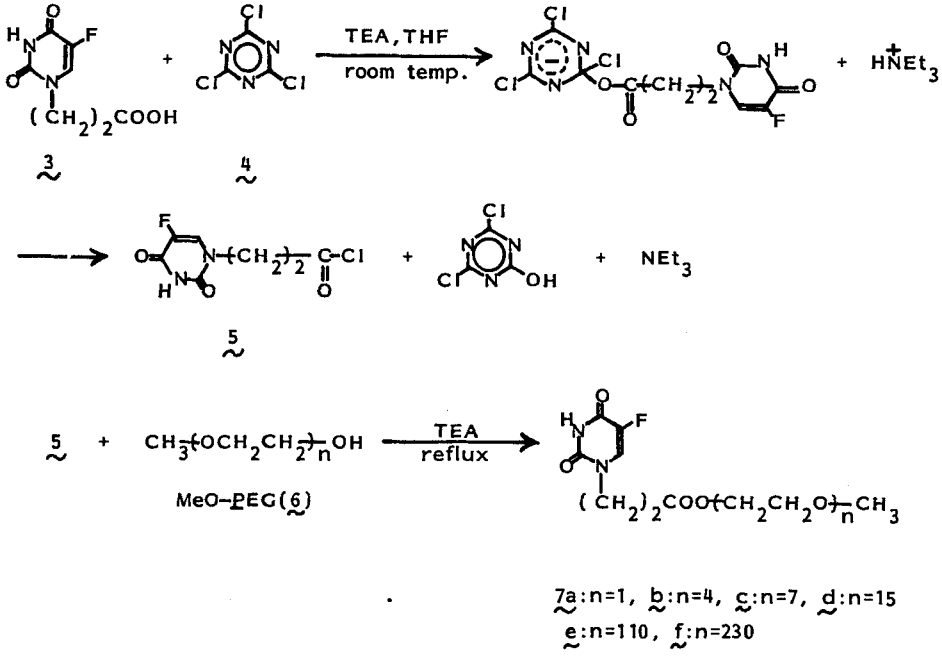
[a]The values of n indicate the degree of polymerization of PEG.
[b]The yields were calculated from the weights of **7**, **11**, and **14** isolated by column chromatography.
[c]Analysis. Calculated for C, 46.16; H, 5.04; N, 10.76; F, 7.30%. Found: C, 46.24; H, 5.14; N, 10.72%.
[d]Analysis. Calculated for C, 44.69; H, 4.82; N, 14.89; F, 10.10%. Found: C, 44.73; H, 4.90; N, 14.87%.

analyses. Characterization of compounds **7**, **11**, and **14** are summarized in Table 1.

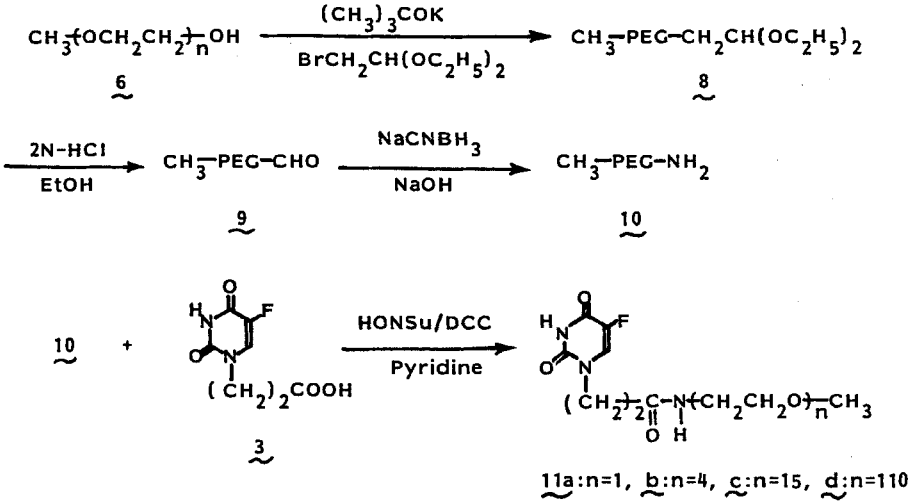
Without isolation of 3-(5-fluorouracil-1-yl) propionyl chloride (**5**) obtained from **3** with cyanuric chloride (**4**), **6** was allowed to react with **5** to give MeO-PEG (n = 1, 4, 7, 15, 110, 230)/ester/1-β-CE-5FU (**7**) through by the reactions shown in Scheme 2.

MeO-PEG (n = 1, 4, 15, 110)/amide/1-β-CE-5FU (**11**) could be obtained from the coupling reaction of **3** with **10** using the active ester method with HONSu/DCC (Scheme 3).

MeO-PEG (n = 1, 4, 15, 110)/ether/1-β-HE-5FU (**14**) could be obtained from the coupling reaction of **12** with **13** in chlorobenzene using tetra-n-butylammonium bromide as a phase-transfer catalyst (Scheme 4).

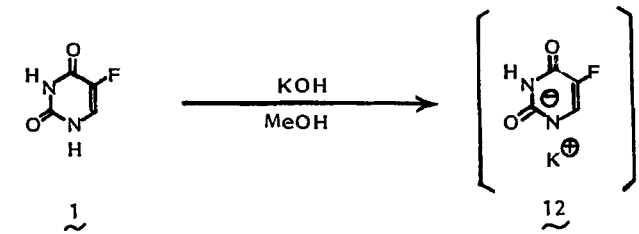


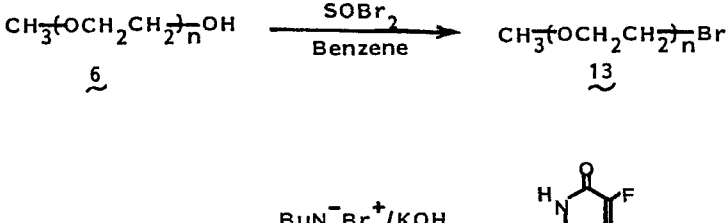
SCHEME 2.

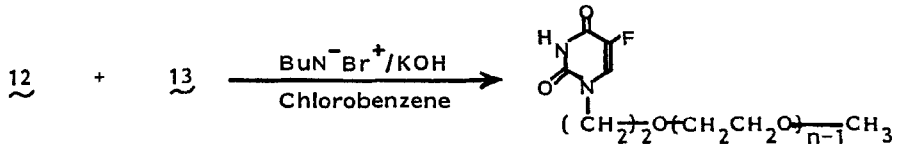


11a:n=1, b:n=4, c:n=15, d:n=110

SCHEME 3.





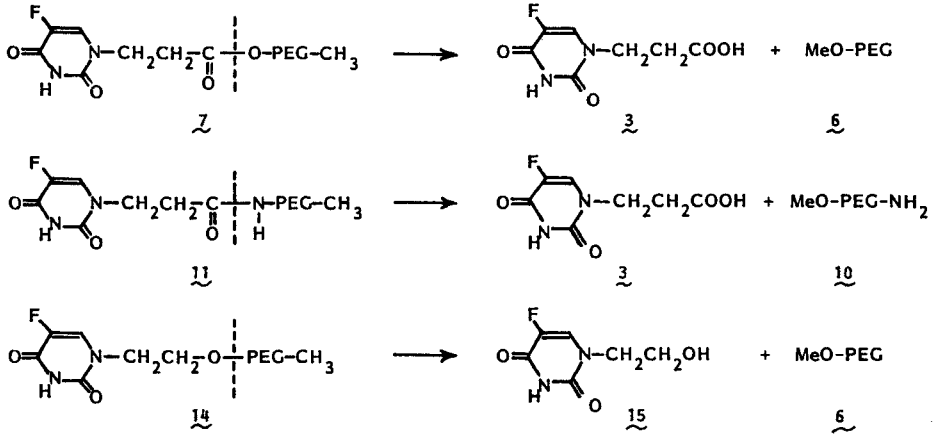


14a:n=1, b:n=4, c:n=15, d:n=110

SCHEME 4.

Hydrolysis of MeO-PEG Compounds with 5FU Units

In order to evaluate the slow release of 5FU or 5FU units from MeO-PEG compounds with 5FU units, the hydrolysis of **7**, **11**, and **14** was investigated *in vitro* at 37°C in the presence or absence of enzymes. The release of 5FU units (**3**, **15**) was measured, but 5FU itself was actually not isolated.



Consequently, the measurement of the amounts of **3** and **15** which were subsequently released was performed by HPLC or GPC.

Systems without Enzyme. The results of the hydrolysis of the ester bond of **7** in 1/15 *M* KH_2PO_4-Na_2HPO_4 buffer solution and in various kinds of aqueous solutions are shown in Figs. 1 and 2, respectively. The ester bond of **7** was found to be hydrolyzed even under mild conditions, such as with phosphate buffer, physiological saline aqueous solutions, or pseudohumor mixture. The hydrolysis rate under alkaline condition was faster than under acidic condition. Moreover, the rate of hydrolysis of the ester bond decreased with increasing *n* in MeO-PEG.

The results of the hydrolysis of the amide bond of **11** in phosphate buffer solution and in other aqueous solutions are shown in Figs. 3 and 4, respectively. Although the tendency of the hydrolysis of the amide bond was the same as that of the ester bond, the rate of hydrolysis of the amide bond was found to be slower.

The results of the hydrolysis of the ether bond of **14** in phosphate buffer solution and in other aqueous solutions are shown in Figs. 5 and 6, respectively.

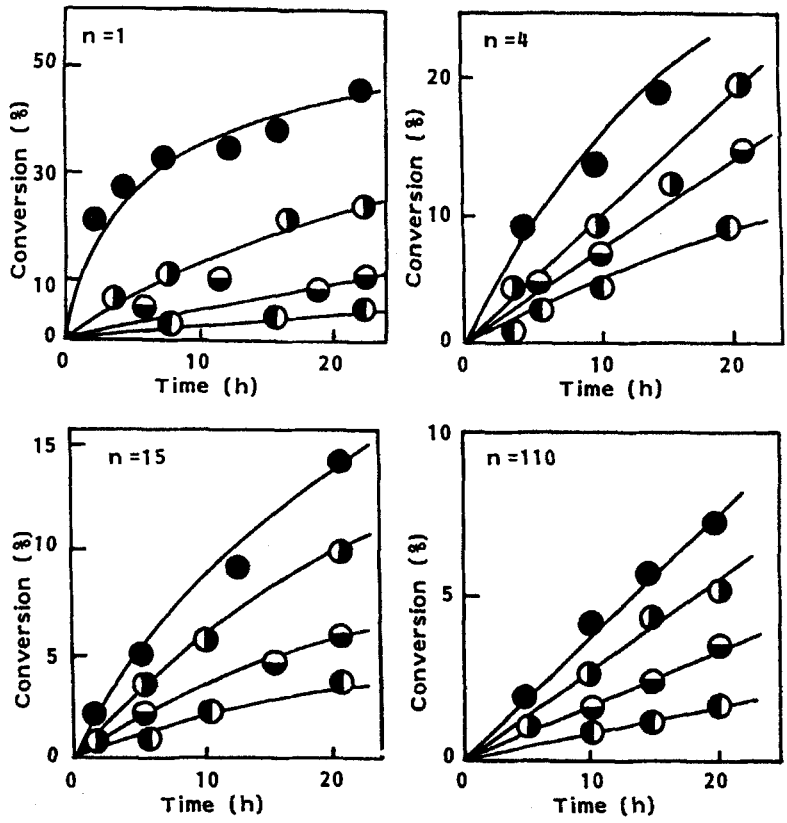


FIG. 1. Time-conversion curves for the hydrolysis of the ester bond of MeO-PEG/ester/1-β-CE-5FU (7) in 1/15 *M* KH_2PO_4-Na_2HPO_4 buffer at 37°C. [S] = 1.53 mmol/L; pH = 7.90 (●), 7.70 (◑), 7.18 (◒), 6.80 (◐).

Generally, the ether bond was found to be hydrolyzed very much less than the ester and amide bonds. Furthermore, the rate of hydrolysis under acidic condition was faster than that under alkaline condition. These results differed from the results obtained when compounds with ester or amide bonds were hydrolyzed. In this case also, the rate of hydrolysis decreased with increasing *n* in MeO-PEG.

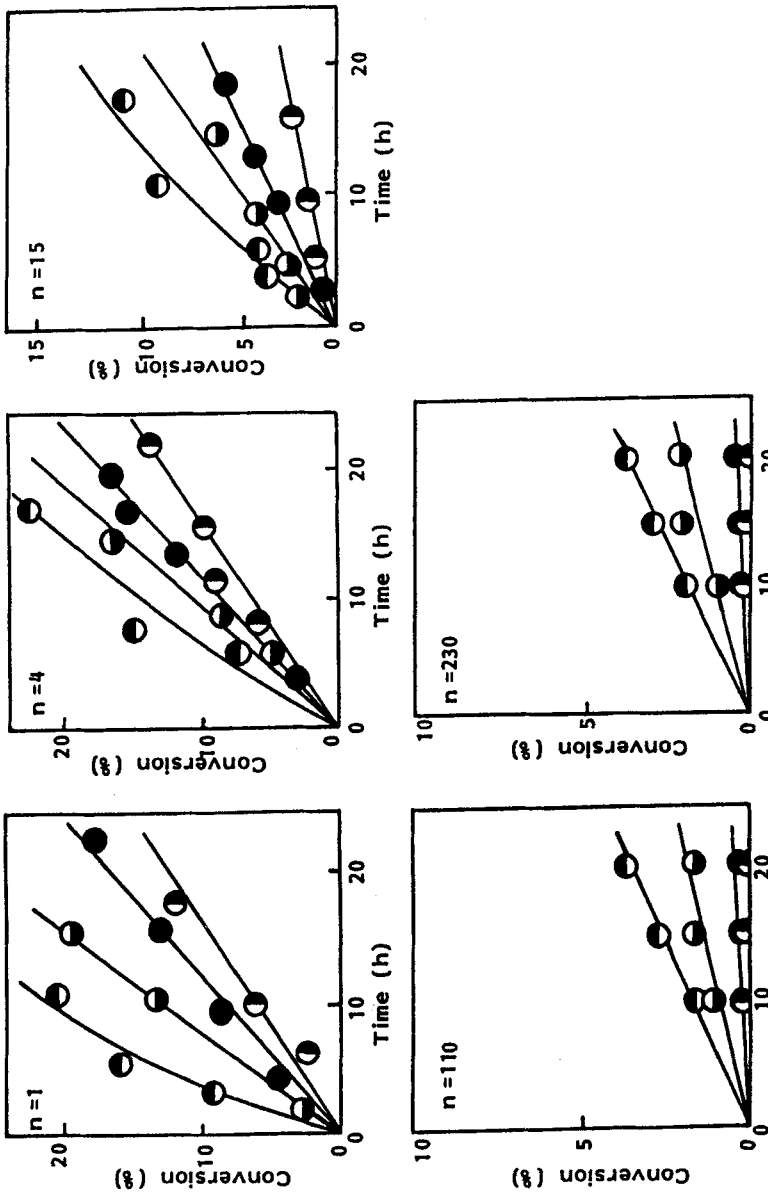


FIG. 2. Time-conversion curves for the hydrolysis of the ester bond of MeO-PEG/ester/1-β-CE-5FU (7) in aqueous at 37°C. [S] = 1.53 mmol/L; (◔) 0.01 *N* NaOH, (◒) 0.1 *N* HCl, (●) physiological saline, (◑) pseudo-humor.

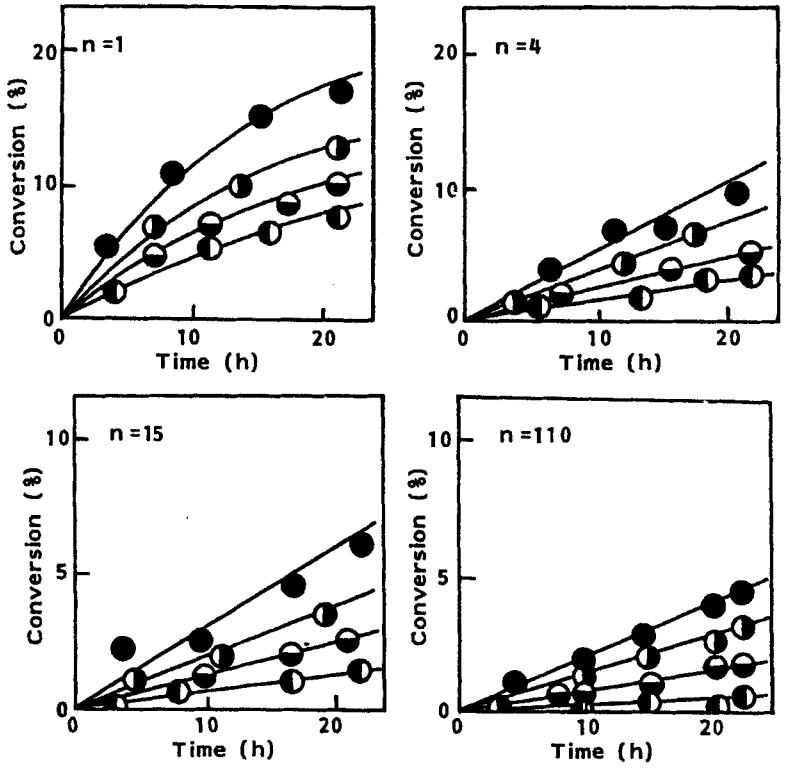


FIG. 3. Time-conversion curves for the hydrolysis of the amide bond of MeO-PEG/amide/1-β-CE-5FU (11) in 1/15 *M* KH_2PO_4-Na_2HPO_4 buffer at 37°C. [S] = 1.53 mmol/L; pH = 7.90 (●), 7.70 (◑), 7.18 (◒), 6.80 (◐).

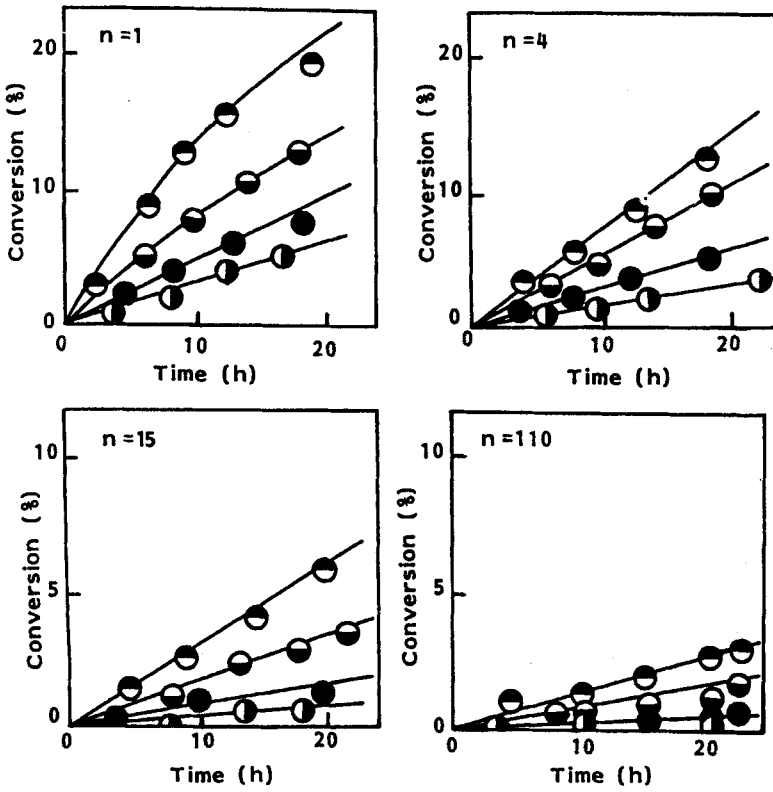


FIG. 4. Time-conversion curves for the hydrolysis of the amide bond of MeO-PEG/amide/1-β-CE-5FU (11) in aqueous solution at 37°C. [S] = 1.53 mmol/L; (◓) 0.01 *N* NaOH, (◒) 0.1 *N* HCl, (●) physiological saline, (◑) pseudo-humor.

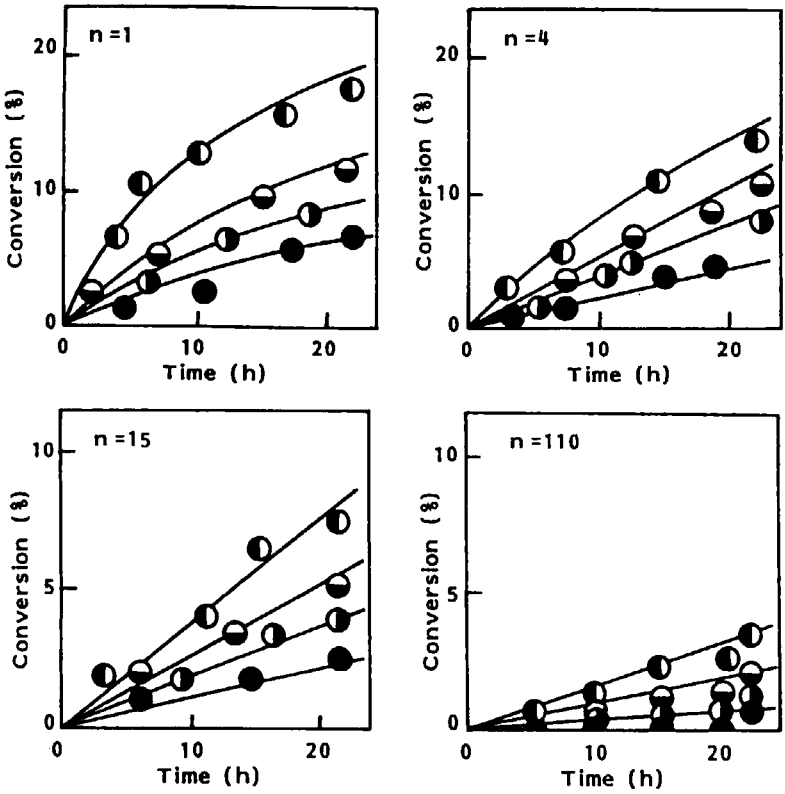


FIG. 5. Time-conversion curves for the hydrolysis of the ether bond of MeO-PEG/ether/1-β-HE-5FU (**14**) in 1/15 *M* KH_2PO_4-Na_2HPO_4 buffer at 37°C. [S] = 1.53 mmol/L; pH 7.90 (●), 7.70 (◑), 7.18 (⊖), 6.80 (◐).

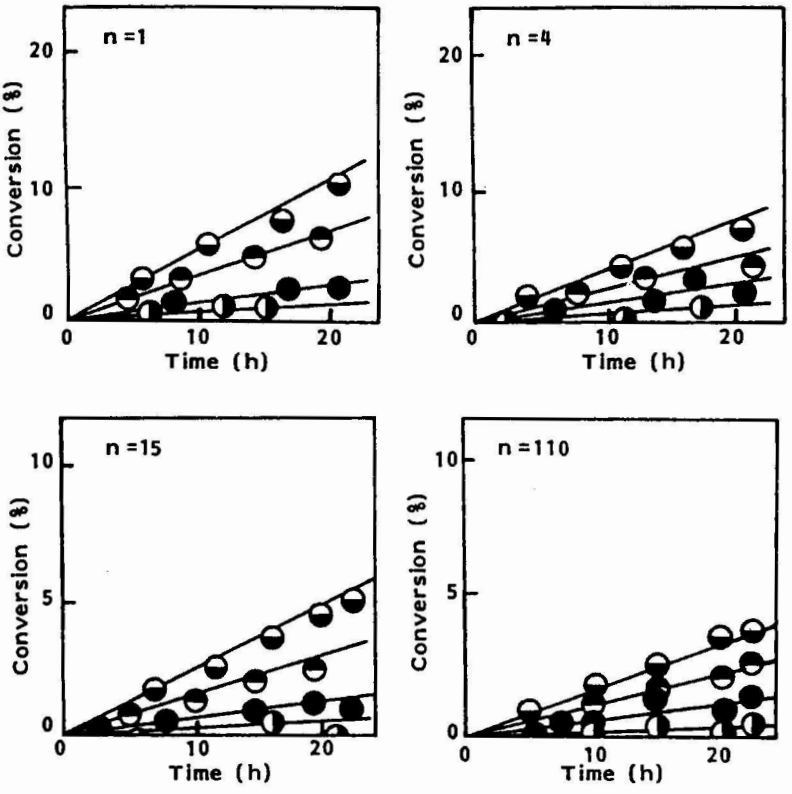


FIG. 6. Time-conversion curves for the hydrolysis of the ether bond of MeO-PEG/ether/1-β-HE-5FU (**14**) in aqueous solution at 37°C. [S] = 1.53 mmol/L; (◓) 0.01 *N* NaOH, (◒) 0.1 *N* HCl, (●) physiological saline, (◑) pseudo-humor.

TABLE 2. Parameters for Hydrolysis[a] in the Presence of Enzymes

MeO-PEG(n)/bond/5FU unit	E, unit·L^{-1}	K_m,[b] mol·L^{-1}	v_{max},[c] mol·L^{-1}·s^{-1}
MeO-PEG(1)/ester/1-β-CE-5FU	0.52×10^4 [d]	1.15×10^{-3}	5.39×10^{-6}
MeO-PEG(4)/ester/1-β-CE-5FU	0.52×10^4 [d]	4.10×10^{-3}	5.66×10^{-7}
MeO-PEG(15)/ester/1-β-CE-5FU	0.52×10^4 [d]	4.36×10^{-4}	2.30×10^{-8}
MeO-PEG(110)/ester/1-β-CE-5FU	0.52×10^4 [d]	1.76×10^{-4}	1.19×10^{-8}
MeO-PEG(1)/amide/1-β-CE-5FU	0.52×10^4 [e]	3.04×10^{-3}	5.01×10^{-6}
MeO-PEG(4)/amide/1-β-CE-5FU	0.52×10^4 [e]	4.02×10^{-3}	4.73×10^{-7}
MeO-PEG(15)/amide/1-β-CE-5FU	0.52×10^4 [e]	5.29×10^{-4}	3.46×10^{-8}
MeO-PEG(110)/amide/1-β-CE-5FU	0.52×10^4 [e]	1.20×10^{-4}	1.01×10^{-8}

[a]In 1/75 M KH_2PO_4-Na_2HPO_4 buffer solution (pH = 7.70) at 37°C.
[b]Michaelis-Menten constant.
[c]Maximum hydrolysis rate.
[d]Esterase was used as the enzyme.
[e]Acylase was used as the enzyme.

System with Enzymes. The ester bond of **7** was hydrolyzed slowly by esterase and α-chymotrypsin but not by lipase. The amide bond of **11** was slowly hydrolyzed by acylase but not by esterase. However, the ether bond of **14** was not at all hydrolyzed by α-amylase. The results of the esterase-catalyzed hydrolysis of the ester bond of **7** and the acylase-catalyzed hydrolysis of the amide bond of **11** are summarized in Table 2, which shows that the ester bond of **7** and the amide bond of **11** were very slowly cleaved by esterase and acylase, respectively.

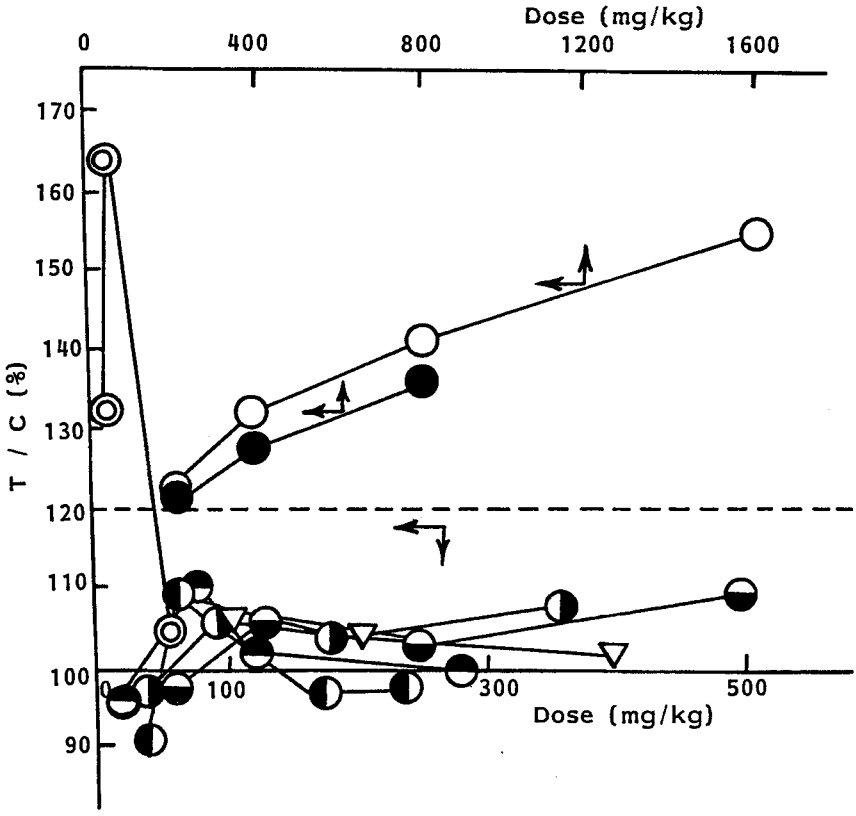


FIG. 7. Plots of antitumor activity vs dose for MeO-PEG/ester/1-β-CE-5FU (**7**). (◐) **7a** (n = 1), (◑) **7b** (n = 4), (⊖) **7c** (n = 7), (⊖) **7d** (n = 15), (○) **7e** (n = 110), (●) **7f** (n = 230), (▽) 1-β-CE-5FU (**3**), (◎) 5FU (**1**).

Antitumor Activity of MeO-PEG Compounds with 5FU Units

The results of the antitumor activity of **7**, **11**, and **14** are shown in Figs. 7, 8, and 9, respectively, and are compared to those of 5FU itself and compounds with 5FU units which are not bound to the end of a PEG chain. Although 5FU units in **3** and **15** exhibit essentially no antitumor activity, MeO-PEG compounds with 5FU units, **7**, **11**, and **14**, show some activities under the same test conditions. Moreover, although the antitumor activity of **7** and **11** increased with increasing *n* in MeO-PEG, the activity of **14** decreased with increasing *n*. These results may be correlated with the difference in the types of unit released from **7**, **11**, and **14**, and with the difference in the state of aggregation of MeO-PEG compounds with 5FU units as a function of *n in vivo*. We will discuss the correlation between the incorporation of 5FU into tumor cells and the state of aggregation of MeO-PEG compounds with 5FU units in a future paper.

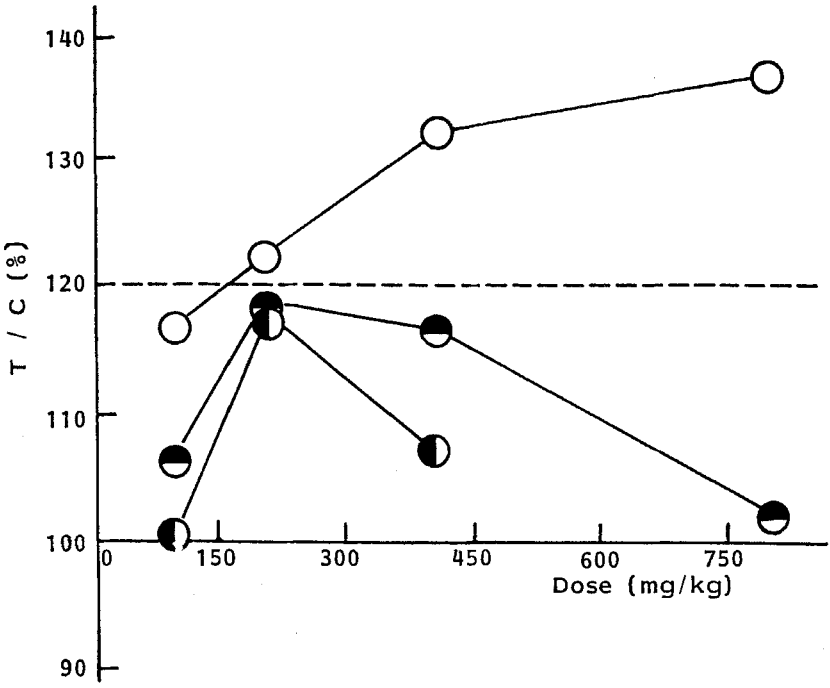


FIG. 8. Plots of antitumor activity vs dose for MeO-PEG/amide/1-β-CE-5FU (**11**). (◐) **11a** (*n* = 1), (◓) **11d** (*n* = 15), (○) **11e** (*n* = 110).

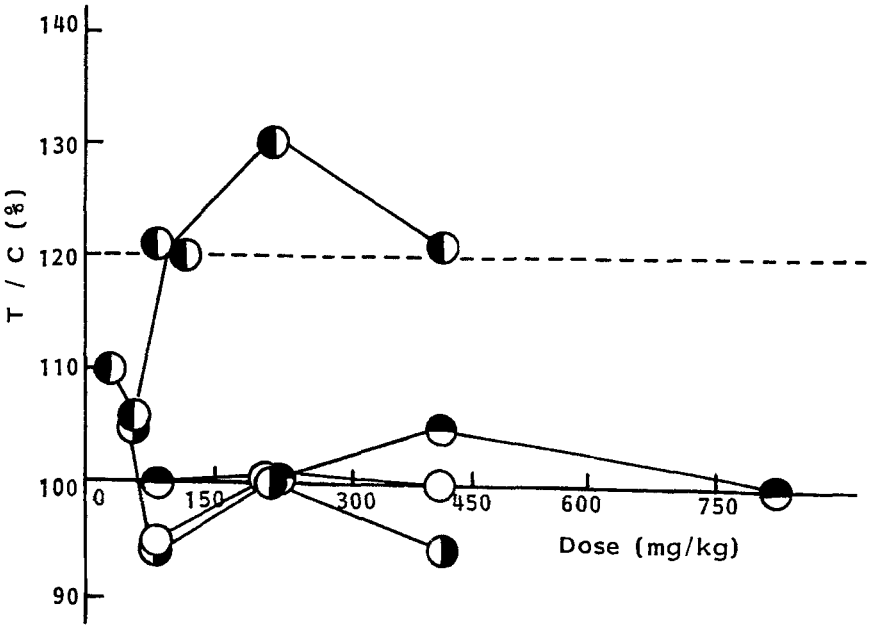


FIG. 9. Plots of antitumor activity vs dose for MeO-PEG/ether/1-β-HE-5FU (**14**). (◐) **14a** (n = 1), (◑) **14b** (n = 4), (◓) **14c** (n = 15), (○) **14d** (n = 110).

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