[Chem. Pharm. Bull. 19(10)2003—2008(1971)]

UDC 547.333.04.057 : 615.277.3.011.5

Studies on Antitumor Substances. XII.[1] Synthesis of Bis(2,3-epoxypropyl)amine Derivatives and the Reaction with Some Nucleophiles

Seigoro Hayashi, Mitsuru Furukawa, Yoko Fujino, Makoto Sugita,[2a] and Toru Nakao[2b]

Faculty of Pharmaceutical Sciences, Kumamoto University[2a] and Yoshitomi Seiyaku Co., Ltd.[2b]

(Received December 25, 1970)

Several bis(2,3-epoxypropyl)amine derivatives were successfully synthesized by the modification of Homer's method. N,N′-Bis(2,3-epoxypropyl)piperazine and *p*-bis(2,3-epoxypropoxy)benzene were attempted to react with thiols, amines and phenol, and the corresponding ring opening compounds of the epoxide ring were obtained in good yields, respectively. N,N′-Bis(2,3-epoxypropyl)piperazine and *p*-bis(2,3-epoxypropoxy)benzene also reacted with diethyl malonate to give N,N′-bis(γ-ethoxycarbonyl-γ-butyrolacton-α-yl)methyl piperazine and *p*-bis(γ-ethoxycarbonyl-γ-butyrolacton-α-yl)methoxy benzene, respectively.

Ross[3] has reported an extensive study on the relationship between the structure of a number of epoxides and their activity against Walker carcinosarcoma 256 in the rat. This study revealed a distinct correlation of the chemical reactivity of the epoxide function toward anions and the observed biological effectiveness. Gerzon[4] has synthesized a number of alicyclic aminobisepoxides, in which some of them, especially N,N′-bis(2,3-epoxypropyl)-piperazine, were found to possess the antitumor activity against leukaemia P 1534 in mice. It is of interest to study the effect of structural modifications in the bisepoxide, particularly of the amine moiety, on the antitumor effectiveness and to investigate the antitumor mechanism, because epoxides have been known to react as electrophilic reagents and demonstrated to react readily with protein at physiological pH.[5]

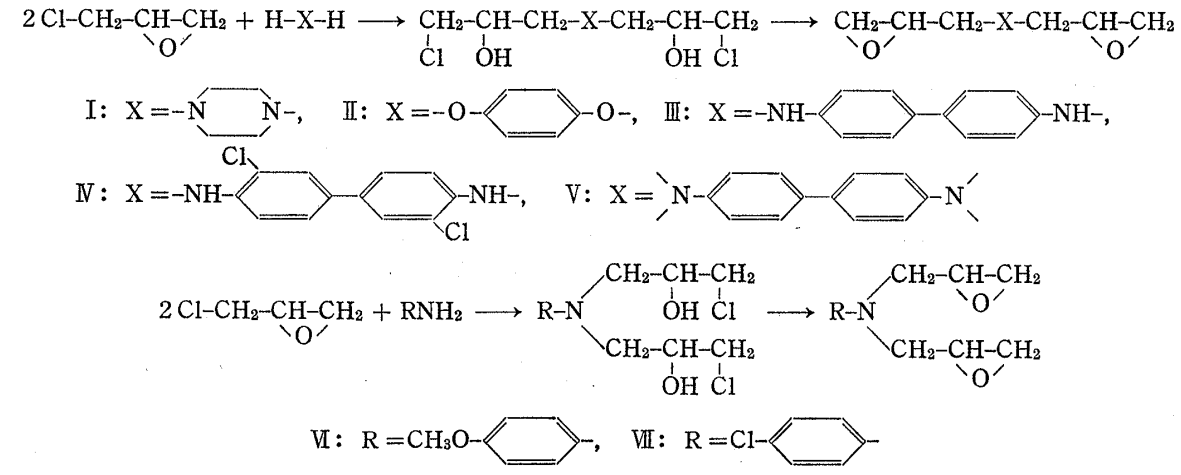
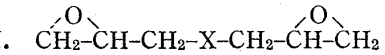
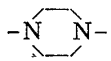
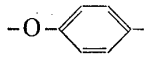
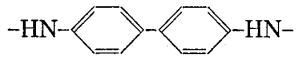
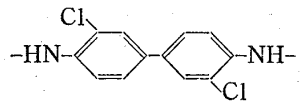
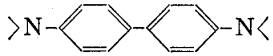
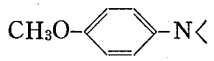
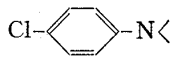
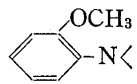
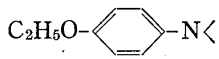


Chart 1

1) Part XI: S. Hayashi, M. Furukawa, Y. Fujino, T. Nakao, and K. Inoue, *Chem. Pharm. Bull.* (Tokyo), **19**, 1557 (1971).
2) Location: *a*) *Oe-moto-machi, Kumamoto* ; *b*) *Yoshitomi-cho, Fukuoka.*
3) W.C.J. Ross, *J. Chem. Soc.*, **1950**, 2257.
4) K. Gerzon, J.E. Cochran, L.A. White, R. Monahan, E.V. Krumkalns, R.E. Scroggs, and J. Mills, *J. Med. Chem.*, **1**, 223 (1959).
5) H.L. Fraenkel-Conrat, *J. Biol. Chem.*, **154**, 227 (1944).

TABLE I. 

No.	X	Yield (%)	mp or bp (°C)	Recryst. Solv.	Appearance	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
I[a]		15.3	63— 64	ether	prisms	$C_{10}H_{22}O_4N$						
II[b]		20.0	118—119	ethyl acetate	needles	$C_{12}H_{14}O_4$						
III		33.5	148—150	dioxane	needles	$C_{18}H_{20}O_2N_2$	72.97	6.80	9.44	72.57	7.00	9.22
IV		31.6	132—133	benzene	needles	$C_{18}H_{18}O_2N_2Cl_2$	59.18	4.97	7.67	59.00	4.97	7.44
V		10.6	82— 83	ether–ethanol	needles	$C_{24}H_{28}O_4N_2$	70.56	7.08	6.86	70.50	6.94	7.00
VI[c]		22.3	48.5—49	ether	needles	$C_{13}H_{17}O_3N$	66.35	7.28	5.95	66.69	7.44	6.17
VII[c]		29.1	36.5—38	ether–ethanol	needles	$C_{12}H_{14}O_2NCl$	60.11	5.89	5.80	60.19	6.01	5.81
VIII		10.5	131—135/0.05		liquid	$C_{13}H_{17}O_3N$	66.35	7.28	5.95	66.11	7.44	6.36
IX		20.0	160—167/0.05		liquid	$C_{14}H_{19}O_3N$	67.44	7.68	5.62	67.46	7.72	5.86

a) SD.M. Burness and H.O. Bayer, *J. Org. Chem.*, **28**, 2284 (1963)
b) Y. Iwakura, S. Izawa, and F. Hayano, *J. Poly. Scie.*, **4**, 751 (1966)
c) R.F. Homer, *J. Chem. Soc.*, **1950**, 3690, VI: bp 120—140°/0.5, VII: bp 178—180°/0.4

In order to find out new bisepoxides having potential antitumor effects and to reveal their reactivity *in vivo*, several bis(2,3-epoxypropyl)amines possessing aromatic ring substituents were synthesized and attempted to react with nucleophiles, such as thiols, amines, phenols and active methylene compounds. These bisepoxides were successfully obtained by the modification of Homer's method[6] by setting aside a methanolic solution of the free amine (1 mole) and epichlorohydrine (2 moles) for a few days at room temperature, followed by the ring closure of the resulting chlorohydrine to epoxide with the theoretical amount of potassium hydroxide in a mixture of ether and dioxane under reflux for ten hours (Chart 1). Bisepoxides obtained were summarized in Table I. By this procedure, bisepoxides were obtained in fairly pure state, though the yields were comparatively low. Generally it is known that solid epoxides are fairly stable, but liquid ones are less stable. Therefore, it is often observed to be difficult to purify by distillation. For example, Homer has reported that difficulty was experienced in obtaining *p*-chloro-N,N-bis(2,3-epoxypropyl)aniline (ref. bp 178—180°/0.4 mm) by distillation in analytically pure state. However, the same compound was obtained in the form of crystals by our procedure in about three times of the yield and the analysis agreed with the theoretical value. These bisepoxides exhibited the characteristic infrared (IR) absorptions of the epoxide ring at near 1250 cm^{-1}, 890 cm^{-1} and 830 cm^{-1}. The nuclear magnetic resonance (NMR) spectra showed multiplets due to the methine hydrogen at near τ 6.50 and multiplets assignable to the methylene hydrogen of the epoxide ring at near τ 7.00.

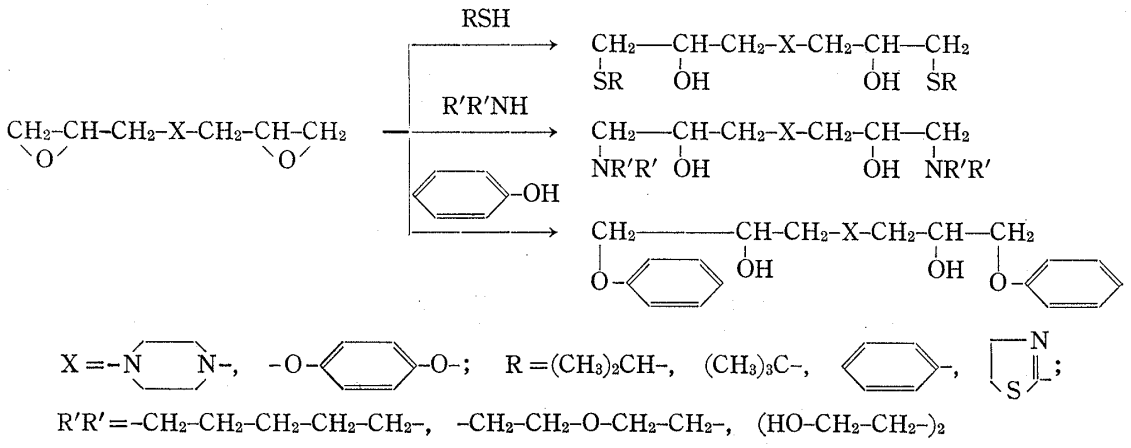
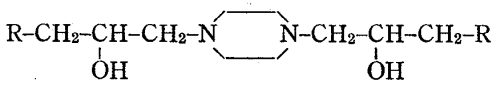
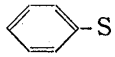
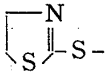
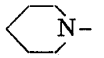
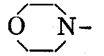
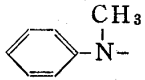
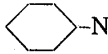
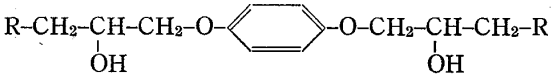
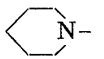
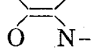
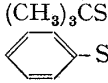


Chart 2

Two bisepoxides, N,N'-bis(2,3-epoxypropyl)piperazine (I) and *p*-bis(2,3-epoxypropoxy)-benzene (II) were attempted to react with thiols, amines, phenol and diethyl malonate as an active methylene compound. In the case of N,N'-bis(2,3-epoxypropyl)piperazine (I), the reaction with thiols was successfully carried out by stirring in methanol for five hours at room temperature to give the corresponding N,N'-bis(3-alkylthio-2-hydroxypropyl)piperazine in almost quantitative yields. The reaction with amines and phenol also gave more than 80% yields of the corresponding N,N'-bis(3-substituted amino-2-hydroxypropyl)piperazine and N,N'-bis(3-phenoxy-2-hydroxypropyl)piperazine, respectively, by heating in methanol under reflux. In the case of *p*-bis(2,3-epoxypropoxy)benzene, the reactions with amines and thiols were analogously successfully proceeded by heating in methanol for fifteen hours under reflux to give the corresponding *p*-bis(3-substituted amino-2-hydroxypropoxy) benzene and *p*-bis(3-alkylthio-2-hydroxypropoxy)benzene, respectively, in fairly good yields. Different from the case of N,N'-bis(2,3-epoxypropyl)piperazine, it is noted that heating with phenols

6) R.F. Homer, *J. Chem. Soc.*, **1950**, 3690.

Table II.

No.	R	Yield (%)	mp (°C)	Recryst. Solv.	Appearance	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
												
X	$(CH_3)_3CS$-	94.9	136 —136.5	ligroin	needles	$C_{18}H_{38}O_2N_2S_2$	57.11	10.12	7.40	57.06	9.54	7.43
XI	$(CH_3)_2CHS$-	97.2	85 — 85.5	ligroin	needles	$C_{15}H_{34}O_2N_2S_2$	54.83	9.78	7.99	54.97	9.81	7.98
XII	C_2H_5S-	99.4	90 — 90.5	ligroin	needles	$C_{14}H_{30}O_2N_2S_2$	52.15	9.37	8.68	51.71	9.27	8.81
XIII		97.5	138 —138.5	EtOH	needles	$C_{22}H_{30}O_2N_2S_2$	63.14	7.23	6.70	63.34	7.50	6.77
XIV		64.5	149 —150.5	MeOH	plates	$C_{16}H_{28}O_2N_4S_4$	44.00	6.46	12.83	43.61	6.56	12.67
XV		86.8	111.5—112	ligroin	needles	$C_{20}H_{40}O_2N_4$	65.18	10.94	15.20	65.00	10.84	14.87
XVI		94.1	125 —125.5	EtOH	needles	$C_{18}H_{36}O_4N_4$	58.03	9.78	15.04	57.52	9.66	14.74
XVII		38.8	143.5	MeOH	needles	$C_{24}H_{36}O_2N_4$	69.86	8.80	13.58	69.58	8.86	13.39
XVIII	$(HOCH_2CH_2)_2N$-	97.3	120 —121	EtOH	needles	$C_{18}H_{40}O_6N_4$	52.92	9.87	13.71	52.60	9.84	13.43
XIX		80.9	159 —159.5	ligroin+EtOH	needles	$C_{22}H_{44}O_2N_4$	66.62	11.18	14.13	66.16	11.17	13.91
XX	$(CH_3CH_2CH_2)_2N$-	90.0	87 — 88	EtOH+H_2O	needles	$C_{22}H_{48}O_2N_4$	66.00	12.08	13.99	65.69	11.86	13.85
XXI		20.7	143.5—144	EtOH	plates	$C_{22}H_{30}O_4N_2$	68.36	7.82	7.25	68.10	7.95	7.17
												
XXII		91.9	112 —112.5	ligroin	needles	$C_{22}H_{36}O_4N_2$	67.31	9.24	7.14	67.02	9.29	7.13
XXIII		90.9	136 —137	benzene	needles	$C_{20}H_{32}O_6N_2$	60.59	8.21	7.07	60.45	8.13	7.15
XXIV	$(CH_3)_3CS$-	84.6	77 — 79	ligroin+toluene	needles	$C_{20}H_{34}O_4S_2$	59.66	8.51		59.29	8.42	
XXV		97.3	98	ligroin+EtOH	needles	$C_{24}H_{26}O_4S_2$	65.10	5.92		65.56	5.84	

resulted in the recovery of the materials. Isaacs[7-9] has shown that two possible orientations should be in the ring opening reaction of epoxides and concluded that one of the main factors of determing the orientation is steric hindrance. However, the formation of the isomeric products was not observed in the reactions mentioned above, probably because bulky nucleophiles were used in all reactions. The compounds obtained were summarized in Table II.

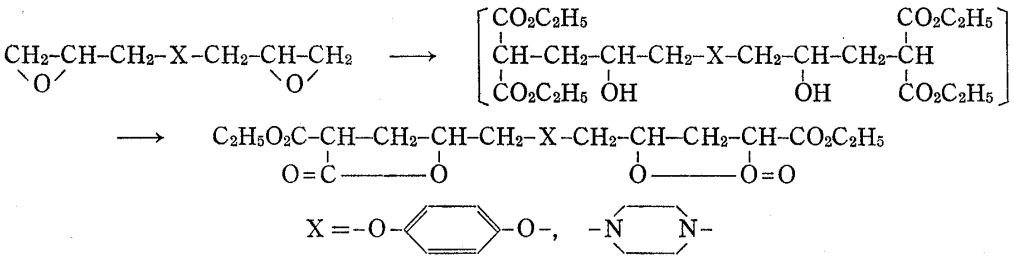


Chart 3

The reaction of *p*-bis(2,3-epoxypropoxy)benzene with diethyl malonate in the presence of sodium ethoxide catalyst was found to give *p*-bis[(γ-ethoxycarbonyl-γ-butyrolacton-α-yl)-methoxy]benzene in 37% yield, probably through the formation of the intermediate shown in Chart 3. The elemental analysis of *p*-bis[(γ-ethoxycarbonyl-γ-butyrolacton-α-yl)methoxy]-benzene was in agreement with that of $C_{22}H_{28}O_{10}$. The IR spectrum exhibited the absorption assignable to the lactone carbonyl group at 1771 cm^{-1} and due to the ester carbonyl group at 1730 cm^{-1}. The NMR spectrum exhibited triplet assignable to methyl hydrogens at τ 8.77, multiplet due to methylene hydrogens between two methin groups at τ 7.60—7.20 and multiplet assignable to another methylene and methin hydrogens at τ 6.40—5.67. Aromatic ring hydrogens appeared as singlet at τ 3.33. The mass spectrum showed M^+ 452. Analogously, N,N′-bis(2,3-epoxypropyl)piperazine reacted with diethyl malonate to give N,N′-bis[(γ-ethoxycarbonyl-γ-butyrolacton-α-yl)methyl]piperazine. The NMR spectrum of this compound was indicative of the existence of ethoxycarbonylbutyrolactonylmethyl grouping ($-CH_2-CH-CH_2-CH-COOC_2H_5$). Signals for the ethoxycarbonyl hydrogens appeared as triplet at τ 8.70 to CH_3 and qualtet at τ 5.84 to CH_2. Methin hydrogen between two methylene groups appeared as multiplet at τ 6.67—6.26. Another methin and methylene hydrogens appeared as multiplet at τ 7.62—7.27. The presence of carbonyl groups of lactone and ester were also exhibited at 1765 cm^{-1} and 1731 cm^{-1} in the IR spectrum. The mass spectrum indicated M^+ 426.

The antitumor activity of bis(2,3-epoxypropyl)amine derivatives synthesized will be reported in another paper.

Experimental

N,N′-Bis(2,3-epoxypropyl)benzidine (III)——A solution of 0.1 mole of benzidine and 0.2 moles of epichlorohydrine in 200 ml of MeOH was stirred for 2 days at room temperature. The reaction mixture was cooled and the crystals deposited were filtered and recrystallized from MeOH to give 41.8% of N,N′-bis-(3-chloro-2-hydroxypropyl)benzidine melting at 134—136°. A mixture of 0.01 mole of N, N′-bis(3-chloro-2-hydroxypropyl)benzidine and 0.02 mole of KOH in a mixture of 50 ml of ether and 10 ml of dioxane was heated for 10 hr under reflux. The mixture was filtered and the filtrate was concentrated. Colorless needles deposited on cooling was recrystallized from dioxane to give 80.2% (overall 33.5%) of N,N′-bis(2,3-epoxypropyl)benzidine melting at 148—150°. *p*-Methoxy-N,N-bis(2,3-epoxypropyl)aniline (VI) was also synthesized by this procedure.

7) N.S. Isaacs and R.E. Parker, *J. Chem. Soc.*, **1959**, 1925.
8) R.E. Parker and N.S. Isaacs, *Chem. Rev.*, **59**, 737 (1959).
9) N.S. Isaacs and R.E. Parker, *J. Chem. Soc.*, **1960**, 3497.

***p*-Chloro-N,N-bis(2,3-epoxypropyl)aniline (VII)**——A solution of 0.5 mole of *p*-chloroaniline and 1 mole of epichlorohydrine in 1 liter of MeOH was stirred for 2 days at room temperature. The solution was concentrated under reduced pressure and cooled with ice. Crystals deposited were recrystallized from a mixture of ligroin and EtOH to give 66.2% of *p*-chloro-N,N-bis(3-chloro-2-hydroxypropyl)aniline melting at 101—102.5°. A mixture of 0.1 mole of *p*-chloro-N,N-bis(3-chloro-2-hydroxypropyl)aniline and 0.2 mole give 44.0% in 50 ml of ether containing 10 ml of dioxane was treated by the procedure described above to of KOH (overall 29.1%) of *p*-chloro-N,N-bis(2,3-epoxypropyl)aniline melting at 148—150°.

o,o'-Dichloro-N,N'-bis(2,3-epoxypropyl)benzidine (IV) and N,N'-tetrakis(2,3-epoxypropyl)benzidine (V) were also prepared by this procedure.

N,N'-Bis(3-alkylthio-2-hydroxypropyl)piperazine (X—XIV)——A solution of 0.005 mole of N,N'-bis(2,3-epoxypropyl)piperazine dihydrate and 0.015 mole of alkylthiol in 20 ml of MeOH was stirred for 4 hr at room temperature. The solution was concentrated under reduced pressure and the precipitates deposited on cooling were filtered and recrystallized from a suitable solvent. Details of the data were illustrated in Table II.

N,N'-Bis(3-substituted amino-2-hydroxypropyl)piperazine (XV—XX)——A solution of 0.005 mole of N,N'-bis(2,3-epoxypropyl)piperazine dihydrate and 0.01 mole of amine in 20 ml of MeOH was heated for 5 hr under reflux. The solution was concentrated under reduced pressure and the precipitates deposited on cooling were filtered and recrystallized from a suitable solvent. Details of the data were shown in Table II.

N,N'-Bis(3-phenoxy-2-hydroxypropyl)piperazine (XXI)——A solution of 0.99 g (0.005 mole) of N,N'-bis(2,3-epoxypropyl)piperazine dihydrate and 0.94 g (0.01 mole) of phenol in 20 ml of MeOH was heated for 5 hr under reflux. The solution was concentrated under reduced pressure and the precipitates deposited on cooling were filtered and recrystallized from EtOH to give 0.40 g (29.1%) of colorless plates melting at 143—144°. *Anal.* Calcd. for $C_{22}H_{30}O_4N_2$: C, 68.36; H, 7.82; N, 7.25. Found: C, 68.10; H, 7.95; N, 7.17.

***p*-Bis(3-alkylthio-2-hydroxypropoxy)benzene and *p*-Bis(3-substituted amino-2-hydroxypropoxy)benzene (XXII—XXV)**——A solution of 0.01 mole of *p*-bis(2,3-epoxypropoxy)benzene and 0.15 mole of alkylthiol or amine in 20 ml of MeOH was heated for 15 hr under reflux. The solution was concentrated and the precipitates deposited on cooling were recrystallized from a suitable solvent. Details of the data were shown in Table II.

***p*-Bis[(γ-ethoxycarbonyl-γ-butyrolacton-α-yl)methoxy]benzene (XXVI)**——A mixture of 0.01 mole of *p*-bis(2,3-epoxypropoxy)benzene, 0.02 mole of diethyl malonate and 0.02 mole of sodium ethoxide in 20 ml of dehyd. EtOH was stirred for 10 hr at room temperature. The solution was acidified with acetic acid on cooling and concentrated under reduced pressure. The residue was poured into H_2O and the precipitates deposited were filtered and recrystallized from EtOH to give 36.7% of colorless prisms melting at 134—136°. *Anal.* Calcd. for $C_{22}H_{28}O_{10}$: C, 58.65; H, 5.82. Found: C, 58.61; H, 5.79. IR ν_{max}^{KBr} cm^{-1}: 1771 (lactone), 1730 (ester). NMR τ: 8.77 (6H, triplet, $J=12$ cps, CH_3), 7.60—7.20 (4H, multiplet, CH_2), 6.40—5.67 (12H, overlap of four methylene and four methin hydrogens), 3.33 (4H, singlet, aromatic ring). Mass Spectrum *m/e*: 452 (M^+).

N,N'-Bis[(γ-ethoxycarbonyl-γ-butyrolacton-α-yl)methyl]piperazine (XXVII)——A solution of 0.02 mole of diethyl malonate and 0.02 mole of sodium ethoxide in 20 ml of dehyd. EtOH was stirred for 30 min. To the solution was added 0.01 mole of N,N'-bis(2,3-epoxypropyl)piperazine dihydrate and the solution was stirred for ten hr at room temperature. After acidification with acetic acid on cooling, the solution was evaporated under reduced pressure. The residue was poured into H_2O and the precipitates deposited was recrystallized from EtOH to give 4.6% of colorless prisms melting at 141.5—142°. *Anal.* Calcd. for $C_{20}H_{32}O_8N_2$: C, 56.31; H, 7.09; N, 6.57. Found: C, 56.40; H, 7.12; N, 6.49. IR ν_{max}^{KBr} cm^{-1}: 1765 (lactone), 1731 (ester). NMR τ: 8.70 (6H, triplet, $J=13$ cps, CH_3), 7.62—7.27 (10H, multiplet, overlap of four methylene and two methin hydrogens), 6.67—6.26 (2H, multiplet, CH), 5.84 (4H, qualtet, $J=13$ cps, CH_2). Mass Spectrum *m/e*: 426 (M^+).

Acknowledgement The authors wish to thank Mrs. K. Shiraki and Yoshitomi Seiyaku Co. Ltd. for microanalytical data and Miss M. Sato for the measurements of IR spectra.