layer was separated dried, filtered, and evaporated. Flash chromatography of the residue (Et_2O/hexane, 2:8) afforded **7** (2.19 g, 70% yield) as an oil: IR 3000, 1200 cm^{-1}; NMR δ 6.78 (3 H, m, Ar *H*), 4.46 (1 H, t, *J* = 6, C*H*$(OEt)_2$), 3.78 (6 H, s, OCH_3), 3.6–3.3 (4 H, m, OCH_2), 2.60 (2 H, t, *J* = 6.6, Ar CH_2), 1.3 (16 H, br s), 1.19 (6 H, t, J = 6 Hz, CH_3). Anal. ($C_{22}H_{38}O_4$) C, H.

5-[9-(2,3-Dihydroxyphenyl)nonyl]-3-methylene-2-dihydrofuranone (1). A solution of BBr_3 (13.50 mL, 13.5 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise at −78 °C under argon to a solution of **7** (1.64 g, 4.5 mmol) in dry CH_2Cl_2 (10 mL). After 3 h of stirring at −78 °C, ether (10 mL) and water (15 mL) were added, and the resulting solution was filtered. The ether layer was separated, dried, filtered, and evaporated to give a viscous oil. This oil was dissolved in THF (2 mL) and added to a saturated NH_4Cl solution (5 mL) containing Zn (0.35 g, 5.4 mmol) and ethyl (bromomethyl)acrylate (**3**; 1.04 g, 5.4 mmol). The resulting mixture was heated at 60 °C for 4 h and then cooled to room temperature. Ether (30 mL) was added and the ether layer separated. To this layer was added *p*-TsOH (0.10 g, 0.6 mmol) and the resulting solution was stirred at 25 °C for 2.5 h. After evaporation, the yellow oil was chromatographed (Et_2O/hexane, 7:3) to yield crystalline bihapten 1 (0.70 g, 47% yield): IR 3620, 3600–3200, 1755 cm^{-1}; NMR δ 6.60 (3 H, br, s, Ar *H*), 6.14 (1 H, m, C=C*H*), 5.65 (1 H, m, C=C*H*), 5.37 (2 H, br s, O*H*), 4.46 (1 H, m, C*H*O), 2–4 (4 H, m), 1.22 (16 H, br s); mp 58–60 °C. Anal. ($C_{20}H_{28}O_4$) C, H.

5-[9-(2,3-Dimethoxyphenyl)nonyl]-3-methylene-2-dihydrofuranone (8). A solution of **6** (2.51 g, 8.5 mmol) in CH_2Cl_2 (3 mL) was rapidly added to a suspension of CrO_3 (5.12 g, 51.0 mmol) in CH_2Cl_2 (25 mL) containing pyridine (8.29 mL) at 25 °C. After stirring for 15 min, the reaction mixture was filtered through silica gel (15 g) and washed with AcOEt (100 mL). The clear pale filtrates were combined and evaporated. The residue was dissolved in THF (4 mL) and added to a saturated NH_4Cl solution (10 mL) containing Zn (0.67 g, 10.2 mmol) and **3** (1.98 g, 10.2 mmol). The resulting mixture was heated at 60 °C for 4 h before being cooled. Ether (50 mL) was added and the ether layer separated. To this layer, *p*-TsOH (0.20 g, 1.2 mmol) was added and the solution was stirred at 25 °C for 2.5 h. After evaporation, the oil was chromatographed (Et_2O/hexane, 5:5) to give **8** as an oil (1.48 g, 48% yield): IR 1755, 1205 cm^{-1}; NMR δ 6.74 (3 H, m, Ar H), 6.11 (1 H, m, C=C*H*), 5.52 (1 H, m, C=C*H*), 4.54 (1 H, m, C*H*O), 3.79 (6 H, s, OCH_3), 3.0–2.5 (4 H, m), 1.28 (16 H, br s). Anal. ($C_{22}H_{32}O_4$) C, H.

Biological Assays. Albino Himalayan spotted Füllingsdorf (from Hoffman-La Roche, Basel) female guinea pigs weighing from 300 to 500 g were sensitized as described by Klečak.[22] On alternate days, the hapten, emulsified in Freund's complete adjuvant (FCA), was injected intradermally (0.1 mL) in the shaved nuchal region of the animal (in all, three injections, five after boost). After 15 days of rest, the elicitation was conducted by an open epicutaneous test (OET): 25 μL of a 4:1 acetone/olive oil solution of hapten was deposited on the shaved flank of the animal (on a 2-cm^2 surface with a standard circular stamp). Tests were read at the 48th hour, using the following scale: 0 = no reaction, 0.5 = slight erythema not covering the whole test area, 1 = erythema covering all the test area, 2 = erythema plus swelling of the test area, 3 = erythema plus swelling going well beyond the test area. Before any sensitization, irritation thresholds (primary toxicity) were determined on FCA-injected controls (same procedure as above for elicitation). Concentrations up to 2% in 4:1 acetone/olive oil of Bihapten were nontoxic. Control groups of eight animals (FCA treated) were used in each experiment.

Registry No. 1, 104876-09-7; **2**, 86-51-1; **3**, 17435-72-2; **3** (acid), 72707-66-5; **4**, 104876-10-0; **5**, 104876-11-1; **6**, 104876-12-2; **6** (adlehyde), 104876-13-3; **7**, 104876-14-4; **7** (adlehyde) (deprotected), 104876-15-5; **8**, 104876-16-6.

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Preparation and Antitumor Activity of New Mitomycin A Analogues

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A series of 26 mitomycin A analogues including 23 new ones was prepared by a variety of methods. The most useful methods were alkoxide exchange on mitomycin A and treatment of 7-hydroxymitosane with 3-substituted 1-phenyltriazenes. Many of the new analogues were superior to mitomycin C in the P388 leukemia assay and the more stringent subcutaneous B16 melanoma assay both in mice. Four of them gave long-term survivors in the latter assay. Quantitative correlations between log *P* and antitumor activity were not possible, but some guidelines for future analogue development are proposed.

Although hundreds of mitomycin C analogues (7-aminomitosanes) have been prepared and tested for antitumor activity, only a small number of mitomycin A analogues (7-methoxymitosanes) are known. The latter include naturally occurring compounds mitomycin A (1),[1] mitomycin F (N^{1a}-methylmitomycin A),[2] and a group of 16 semisynthetic analogues.[3] Of the semisynthetic compounds, 14 had the 7-methoxy group replaced by other simple alkoxy groups. All 16 were active against sarcoma-180 in mice. There are a few other naturally occurring 7-methoxy compounds in the mitomycin family, but they are placed in other subgroups.[2] For example, mitomycins B and J (B group) are epimeric at C-9 from the mitomycin A group, and mitomycins H and K (G group) have a 9,10-methylene group.

Our interest in mitomycin A analogues is based on the group of semisynthetic compounds plus our observation that mitomycin A is highly potent (in terms of minimal effective dose, MED) against P388 leukemia in mice.[4] Thus, it is 4 times as potent as mitomycin C, although it is less effective in prolonging life than mitomycin C at their optimal doses (OD). As described below, we prepared a series of 26 mitomycin A analogues, including 23 new ones that were different from the simple 7-alkoxy types. They contained such functionalities as double and triple bond,

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(3) Urakawa, C.; Nakano, K.; Imai, R. *J. Antibiot.* **1980**, *33*, 804.
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alcohol and ether, chlorine, sulfur, tertiary amine, and heterocyclic rings.

Chemistry. At the start of this investigation there were two known methods for preparing 7-alkoxymitosanes: treatment of a 7-hydroxymitosane[5] with a diazoalkane, which had not been investigated beyond the use of diazomethane to make mitomycin A,[5] and alkoxide exchange with mitomycin A, which had been used for the previous series of analogues. We first investigated the scope of the diazoalkane reaction by treating either solutions of 7-hydroxymitosane with appropriate diazoalkanes. This procedure (method A) gave satisfactory yields of mitomycin A (1) and 7-ethoxymitosane (2), but it was not useful for larger diazoalkanes.

A method discovered accidentally was the formation of amino imidates when 7-hydroxymitosane was treated with carbodiimides (method D). The initial purpose of this procedure was to prepare mitomycin C analogues by using carbodiimides and amines, based on an analogy between the hydroxyquinone (weak acid feature of 7-hydroxymitosane) and carboxylic acids. However, the amino imidate (27) formed from dicyclohexylcarbodiimide proved to be very stable and unreactive toward amines, even when heated with excess amine. The small number of carbodiimides available severely limits this method.

Most of the compounds described herein were prepared by alkoxide exchange (method B). In this method, mitomycin A is treated with potassium hydroxide in the new alcohol as solvent.[3] The process is reversible and driven by the mass effect of the solvent, unless the solvent anion is significantly poorer as a nucleophile or better as a leaving group than methoxide. This method does not work with solid alcohols because their solutions in inert solvent lose both the mass effect and stabilization of the polar transition state. Polyols such as ethylene glycol and glycerol present problems because of their viscosity and the difficulty in separating them from the products. Twenty of the compounds in Table I, including 18 new ones, were prepared by alkoxide exchange. They contained various functional groups including halogen, olefin, acetylene, alcohol, ether, sulfide, disulfide, and tertiary amine. Primary and secondary amines could not be used because they would give 7-amino (mitomycin C) analogues.

During the course of this investigation a useful new method for 7-alkoxymitosanes was developed by Vyas and co-workers.[6] This method involves treating 7-hydroxymitosane with a 3-alkyl-1-aryltriazene in an inert solvent such as ether or methylene chloride. Although only six of the compounds in Table I were made by this procedure (method C), it appears to be general and the method of choice in many cases. There is concern about possible reaction of the 7-alkoxymitosane with the aniline liberated on decomposition of the triazene, but this is not really a problem for aniline or *p*-chloroaniline in nonpolar solvent. More practical limitations are availability of the amine, which will be treated with phenyldiazonium hexafluorophosphate to give the triazene, and stability of the triazene. In summary, the 27 compounds, including 23 new ones, in Table I were prepared by a variety of methods, of which only the alkoxide exchange and triazene methods were general.

Antitumor Activity. Table II lists the activity of the mitomycin A analogues against P388 leukemia in mice. This tumor is relatively susceptible to mitomycins and it is the principal database for them.[7] Some of the mitomycin A analogues were also tested in a subcutaneous B16 melanoma assay. In this assay the drug is administered intravenously at a site (tail vein) remote from the tumor, which makes it an especially stringent assay. The results of this assay are also given in Table II. The compounds were not all assayed at the same time, but each experiment contained a mitomycin C control group as well as an untreated group. Consequently, compounds should be compared on the basis of how each one relates to its mitomycin C control, rather than directly. In Table II the median survival time (MST) is given for each compound at its optimal dose (OD), followed by the number of survivors (if any) at the end of the experiment, in brackets, and the MST for mitomycin C at its OD, in parentheses.

For activity against P388 leukemia in mice, Table II reveals that most of the mitomycin A analogues are more potent (MED) than mitomycin C, which usually has a MED of 0.2 mg/kg.[4,7] A few of them (**15**, **21**, and **25**) are 8 times as potent as mitomycin C, and **9**, the 7-hydroxyethoxy analogue, is 32 times as potent. This compound is one of the most potent antitumor agents in mice. Eleven analogues give greater prolongation of life (efficacy) than mitomycin C and five analogues give one or more 30-day survivors. Thus, as a group they are highly potent and efficaceous. The more active members of this group have a variety of functionalities at the 7-position, but most of them involve hydroxyl or ether groups. Against subcutaneous B16 melanoma each of the 14 analogues tested, except **26**, showed substantially better efficacy than mitomycin C, as indicated by at least 41% greater increase in life span (ILS) in one or more experiments. Each analogue except **17** showed greater potency (MED) than mitomycin C, whose MED is in the range 1–3 mg/kg. Compounds **13**, **17**, **21**, and **22** gave long-term survivors. The best among them, **13** and **22**, gave 5/6 and 6/6 long-term survivors, respectively, whereas mitomycin C gave none. Thus, these new mitomycin A analogues constitute an effective group of compounds in this most selective assay. It should be noted that it is difficult to rate the relative activities among the various analogues because the mitomycin C controls were inactive in some experiments. This pertains especially to compounds **9** and **2**, which had good activities even under this condition.

Structure–Activity Relationships. A quantitative correlation between log *P* values (Table II) and the potencies (MED) of compounds against P388 leukemia was attempted, but the result was statistically insignificant. The coefficient of determination R^2 was only 0.10. In this calculation, all compounds except **10** were used ($n = 26$). That compound has a methyl group on the aziridine ring and this feature reduces potency independent of log *P*. The correlation was attempted again using OD instead of MED, but no improvement was obtained. Inclusion of other parameters in the corelation was considered, but it was fruitless. All of the compounds except **27** should have the same quinone reduction potential, which removes this factor from consideration. Inspection of the size of substituents of the compounds (Table I) revealed no obvious relationships between size and antimor activity, and a correlation between MED and RM values was not statistically significant ($R^2 = 0.03$). An attempted correlation between log *P* and activities against subcutaneous B16 melanoma also was unsuccessful. The main difficulty in this case was that the compounds showed little variation

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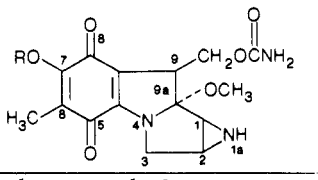
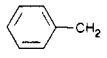
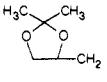
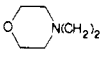
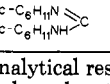
Table I. Preparation and Properties of O^7-Substituted Mitomycin A Analogues[a]



no.	R	method	yield, %	solvent impurity	melt. dec temp, °C	^{1}H NMR signals for the 7-substituent,[b] δ
1	CH_3 (mit. A)	A (ref 5)	50			
2	C_2H_5	A (ref 3)	40			
3	$(CH_3)_2CH$	B (ref 3)	79[c]			
4[d]		B	29	$0.5H_2O$	83–88	3.9–4.4 (m, 3, CH_2O overlapped with signals of one proton of CH_2N), 1.65–2.1 (s, 7)
5[e]	$H_2C{=}CHCH_2$	B	42	$0.25H_2O$	106–111	4.4–4.85 (m, 4, CH_2O overlapped with CH_2OCO), 5.15–5.3 (dd, 1), 5.3–5.5 (dd, 1), 5.8–6.2 (m, 1)
6[f]	$HC{\equiv}CCH_2$	B	31		77–80	4.5–4.9 (m, 4, CH_2O overlapped with CH_2OCO), 2.5 (s, 1)
7		A, B (ref 3)	10, 68			5.32 (s, 2), 6.93–7.63 (s, 1)
8	$Cl(CH_2)_3$	B	64		142–145	2.1–2.35 (m, 2), 3.4–3.8 (t, 2), 4.35–4.5 (t, 2)
9	$HO(CH_2)_2$	B, C	29, 17	$0.5H_2O$	72–74	4.00–4.83 (m, 5, $2CH_2O$ overlapped with signals of a proton of CH_2N)
10[g]	$HO(CH_2)_2$; 1a-CH_3	B	40	$0.3CH_2Cl_2$	80–82	3.97–4.7 (m, 4), 5.4–5.6 (br s, 1); MS MH^+ 394
11[h]	$HO(CH_2)_3$	B, C	23, 21	$0.25H_2O$	80–100	2–2.2 (m, 2), 3.9 (t, 2), 4.25–4.45 (t, 2)
12[i]	$HOCH_2CH(OH)CH_2$	C	10	$1.0H_2O$	above 300	3.3–3.5 (m, 5), 4–4.5 (m, 2)
13	$HO(CH_2)_2O(CH_2)_2$	B	47	$0.5H_2O$	125–128	4.4–4.7 (m, 4, CH_2O-quinone overlapped with CH_2OCO), 3.4–3.85 (m, 9, $3CH_2O$ + OH overlapped with signals of 9-CH and a proton of CH_2N)
14[j]	$HO(CH_2)_2S(CH_2)_2$	B	32		92–95	4.4–4.5 (t, 2), 3.7–3.85 (t, 2), 2.65–3 (t, 4)
15[k]	$HO(CH_2)_2SS(CH_2)_2$	B	44		87–95	4.3–4.8 (m, 4, CH_2O-quinone overlapped with CH_2OCO), 4.4.3 (m, 3, CH_2O overlapped with signals of a proton of CH_2N), 2.5–3 (m, 6, $2CH_2S$ overlapped with azirdine protons signals)
16		B	60		128–133	4.2–4.35 (d, 2), 4–4.2 (m, 1), 3.7–3.9 (t, 2), 1.75–2 (s, 7, $2CH_2$ furan + 6-CH_3)
17[l]		B	31	$0.5H_2O$	68–75	2–2.2 (m, 2), 3.7–4 (m, 4), 5.4–5.6 (m, 1)
18		B	46	H_2O	135–138	1.3–1.6 (s, 6), 3.35–3.75 (m, 4, CH_2O-pyran + 9-CH + a proton of CH_2N), 3.9–4.3 (m, 4, CH_2O-quinone + CHO-pyran + a proton of CH_2N)
19[m]		C	4.3	$0.5H_2O$	110–117	5.45 (s, 2), 6.5 (s, 2), 7.4–7.55 (s, 1)
20[n]		B	30		136–138	1.5 (s, 6), 3.9–4.25 (m, 3, ring CH_2O + a proton of CH_2N), 4.25–4.6 (m, 3, CH_2O-quinone + ring CHO)
21[o]	$(CH_3O)_2CHCH_2$	C	36		68–75	3.4 (s, 6), 4.25–4.3 (d, 2), 4.4–4.9 (m, 5, OCHO + CH_2OCO + NH_2)
22	$CH_3O(CH_2)_2O(CH_2)_2$	B	58		102–104	4.35–4.55 (t, 2, CH_2O-quinone), 3.5–3.85 (m, 8, $3CH_2O$ + a proton of CH_2N + 9-CH), 3.4 (s, 3)
23[p]	$C_2H_5O(CH_2)_2O(CH_2)_2$	B	62	$1.5H_2O$	140–143	4.15 (m, 2, CH_2O-quinone), 3.45–3.9 (m, 11, $4CH_2O$ + CH_2N + 9-CH), 1–1.16 (t, 3)
24	$NC(CH_2)_2$	C	18	$2H_2O$	76–79	2.65–2.8 (t, 2), 4.37–4.5 (t, 2)
25	$(CH_3)_2N(CH_2)_2$	B	71	$0.5H_2O$	140–143	2.25 (s, 6), 2.55–2.65 (t, 2), 4.33–4.45 (t, 2)
26[q]		B	17		136–140	4.25–4.55 (m, 2), 2.6–2.75 (t, 2), 2.4–2.6 (t, 4), 3.35–3.8 (m, 6, $2CH_2O$ of morpholine + 9-CH + a proton of CH_2N)
27[r]		D	50		114–116	0.43–1.87 (m, 22)

[a] Analytical results were within ±0.40% of theoretical value for all elements (C, H, N), except as shown in subsequent footnotes. In some examples solvent impurity (water) had to be added to reconcile the calculated and found values for these elements. Some products were hygroscopic, and although dried under vacuum, they could not be heated because of instability. [b] The solvent was $CDCl_3$ unless specified otherwise. [c] Prepared by the literature method involving sodium isopropoxide. [d] N: calcd, 10.19; found, 9.32. [e] H; calcd 5.67; found, 6.08. [f] N: calcd, 10.86; found, 9.86. [g] H: calcd, 5.64; found, 6.08. [h] N: calcd, 10.56; found, 10.05. [i] H: calcd, 5.85; found, 5.33. [j] H: calcd, 5.90; found, 5.42. N: calcd, 9.19; found, 8.51. [k] N: calcd, 8.91; found, 7.23. [l] N: calcd, 10.14; found, 9.44. [m] N: calcd, 9.90; found, 9.37. [n] N: calcd, 9.35; found, 8.51. [o] N: calcd, 9.92; found, 9.36. [p] H: calcd, 6.69; found, 5.65. [q] N: calcd, 12.50; found, 11.39. [r] N: calcd, 12.92; N, 11.86.

in either the MED or OD values.

Although quantitative correlation failed, some ideas for future analogues can be found in rough qualitative correlations. Thus, if the compounds in Table II are divided into two groups, those that give better prolongation of life than mitomycin C in mice with P388 leukemia and those that do not, the former group contains functionalities with straight chains and/or relatively low lipophilicity (**2**, **9**, **11–15**, **21**, **23**). Among these compounds, the ones with straight chains bearing terminal OH groups were much more active than mitomycin C (ILS > 50%). Compounds with tertiary amine functionalities also have superior ac-

Table II. Antitumor Activity of Mitomycin A Analogues[a]

	intraperitoneal P388 leukemia[b]			subcutaneous B16 melanoma[c]			
no.	max effect, % T/C	opt dose, mg/kg	MED, mg/kg	max effect, % T/C	opt dose, mg/kg	MED, mg/kg	log P[d]
1	180 (270)	3.2	0.05	177 (170)	0.3	0.05	0.26*
2	188 (181)	1.6	<0.05	212 (100)	0.4	<0.4	0.76
				215 (170)	0.2	0.1	
3	174 (268)	3.2	0.2				1.26
4	144 (178)	1.6	0.4				2.26
5	156 (239)	1.6	0.1				0.87
6	150 (239)	0.8	<0.05	204 (147)	0.8	0.2	0.74
7	167 (294)	3.2	<0.2				2.22
8	150 (205)	3.2	0.2				1.65
9	217 (178)	0.2	≪0.1	178 (100)	0.4	0.2	−0.36
	178 (194)	0.4	0.0063				
	>328 [3] (194)[e]	0.4	0.0063				
10	222 (272)	3.2	0.05				0.67
11	175 (160)	1.6	0.05				0.14
12	225 [2] (205)	12.8	0.2				−0.98
13	210 (160)	0.4	0.1	>265 [5] (170)	0.8	0.4	−0.33
14	250 [2] (205)	0.8	<0.1				1.07
15	259 (241)	1.6	<0.025	243 (136)	0.8	0.2	1.35
16	239 [1] (250)	0.8	<0.05	212 (133)	0.8	0.2	1.29
				198 (147)	0.8	0.2	
17	178 (206)	3.2	<0.05	198 [1] (135)	1.6	1.6	0.79
18	161 (178)	0.8	0.1				1.79
19	139 (194)	6.4	6.4				1.60
20	178 (250)	0.8	<0.05	221 (133)	0.6	<0.3	0.82
				198 (147)	0.8	0.4	
				240 (170)	0.6	0.1	
21	269 (263)	1.6	<0.025	216 [3] (162)	1.6	0.4	−0.18
22	225 (230)	0.8	<0.1	>191 [5] (152)	0.8	0.4	0.32
				>265 [6] (170)	0.8	0.4	
23	167 (156)	1.6	<0.1	196 (147)	0.4	0.2	0.82
24	200 (239)	0.4	0.05	141 (100)	1.2	0.8	−0.08
25	281 (263)	3.2	<0.025	220 (162)	1.6	0.4	0.46
26	256 [2] (194)	3.2	<0.05	113 (135)	0.4		0.49
27	150 (238)	51.2	12.8				4.90

[a] Determined at Bristol-Myers Co., Syracuse, NY. [b] A tumor inoculum of 10^6 ascites cells was implanted ip in CDF_1 female mice. Six mice were used at each dose of the mitosane, given once on day 1, and 10 control mice were injected with saline. A control group of six mice at each dose received mitomycin C in the same experiment: MST = median survival time; max effect (% T/C) = (MST treated/MST control) × 100 at the optimal dose (opt dose); MED = minimum effective dose (% T/C = 125). The number of 30-day survivors at the optimal dose is given in brackets beside the maximum effect and the maximum effect of the mitomycin C control at its optimal dose (usually 3.2 or 4.8 mg/kg) in the same experiment is given in parentheses. [c] A tumor inoculum is implanted subcutaneously in BDF_1 female mice. Ten mice were used at each dose of the mitosane, given in three equal parts on days 1, 4, and 7, by intravenous administration in the tail vein. Ten control mice received intravenous saline. Definitions of test result expressions are given above. The number of 60-day survivors at the optimal dose is given in brackets beside the maximum effect and the maximum effect of the mitomycin C control at its optimal dose (1–3 mg/kg) in the same experiment is given in parentheses. [d] Determination by the method of Hansch et al. (Hansch, C.; Muir, R. M.; Fiyita, T.; Malongy, P. P.; Geiger, F.; Struch, M. J. *J. Am. Chem. Soc.* **1963**, *85*, 2817) is indicated by an asterisk. Other log P values are estimated from 1 and values from Hansch and Leo (Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley-Interscience: New York, 1969). [e] Given in equally divided doses on days 1, 4, and 7.

tivity (**25**, **26**). Among these compounds, all that were tested against subcutaneous B16 melanoma, except for **26**, showed activity superior to that of mitomycin C. There were six compounds (**6**, **16**, **17**, **20**, **22**, and **24**) that were more active than mitomycin C against the melanoma, but less active against P388 leukemia. Three of them have straight-chain substituents on O-7 (**6**, **22**, **24**) and the other three are tetrahydrofuran and dioxolane derivatives.

Conclusions. Although mitomycin A analogues have been studied much less than mitomycin C analogues, it is possible to prepare a variety of the former by the triazine or alkoxide exchange methods. These analogues are highly potent (MED) and many of them have better efficacy than mitomycin C against P388 leukemia in mice. They are particularly effective in the stringent subcutaneous B16 melanoma assay. Thus, they represent an important new group of mitomycins and further analogues of this type are clearly warranted. Although it was not possible to derive useful quantitative structure–activity relationships for these compounds, some qualitative guidelines for future analogues are suggested. They include 7-O substituents with a variety of straight chains and low lipophilicity. Cyclic ethers and tertiary amines also appear promising. The preparation and antitumor testing of additional mitomycin A analogues is in progress. Together with the present analogues they will be examined by molecular modeling. Quantitative correlations based on both measured physical properties and calculated binding strengths will be attempted.

Experimental Section

Melting points were recorded on a Mel-Temp melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a JEOL FX90Q (90 MHz) spectrometer and absorptions are reported as downfield from Me_4Si. Elemental analyses were performed by the University of Arizona Analytical Center or Mic Anal, Inc., Tucson, AZ. Analytical values were within ±0.4% of theoretical values unless specified otherwise.

Preparation of Mitomycin A Analogues. Method A. A solution of 7-hydroxymitosane (100 mg)[5] in 50 mL of anhydrous ether was cooled to −10 °C and treated with excess of the freshly prepared diazoalkane in ether or petroleum ether. After 5 h the solvent was removed under reduced pressure and the product was purified by preparative TLC on silica gel (20 × 20 cm, 2-mm thick) with $CHCl_3$–MeOH (8:2) as solvent. The purple band was scraped off and extracted with CH_2Cl_2–MeOH. Removal of solvent gave the products as solids. Their yields and physical properties are recorded in Table I. The diazoalkanes were prepared as follows:

Table III. Chromatographic Solvent Systems for the Products of Method B

compd	solvent system: column	solvent system: preparative TLC
4		Et_2O (repeated)
5		Et_2O (repeated),[a] then $CHCl_3$–acetone (1:1)
6		Et_2O (repeated),[a] then $CHCl_3$–acetone (1:1)
8		$CHCl_3$–MeOH (9:1)
9	$CHCl_3$–MeOH (8:2)	$CHCl_3$–MeOH (9:1)
11	1% MeOH in Et_2O, then $CHCl_3$–MeOH (6:4)	1% MeOH in Et_2O,[a] then $CHCl_3$–MeOH (9:1)
13	10% acetone in Et_2O, then $CHCl_3$–MeOH (6:4)	10% acetone in Et_2O,[a] then $CHCl_3$–MeOH (9:1)
14	6.3% MeOH in Et_2O, then Et_2O–MeOH (8:2 or 7:3)	$CHCl_3$–acetone (9:1)
15	$CHCl_3$–acetone (1:1), then $CHCl_3$–MeOH (9:1)	$CHCl_3$–acetone (3:7)
16	$CHCl_3$–MeOH (19:1)	$CHCl_3$–MeOH (19:1 or 9:1)
17		Et_2O (repeated),[a] then $CHCl_3$–MeOH (9:1)
18	$CHCl_3$, then $CHCl_3$–MeOH (19:1)	$CHCl_3$–MeOH (19:1)
20	$CHCl_3$–acetone (7:3)	$CHCl_3$–acetone (7:3)
22		$CHCl_3$–MeOH (9:1)
23	$CHCl_3$–MeOH (9:1)	$CHCl_3$–MeOH (9:1)
26	Et_2O, then $CHCl_3$–MeOH (9:1)	Et_2O (repeated),[a] then $CHCl_3$–MeOH (19:1)

[a] In these isolations the product (pink) remains on the base line and the excess alcohol and impurities move from it. A second chromatography on TLC in the second solvent moves and further purifies the product.

diazoethane from ethylnitrosourea and KOH;[8] phenyldiazomethane from benzalhydrazone and mercuric oxide.[9]

Method B.[3] A solution of mitomycin A (100 mg, 0.286 mmol) in 2–4 mL of the alcohol was stirred under N_2 for 45 min with 240 mg (**8**, **9**, 11, 14, **15**, **18**, **22**, **25**, **26**) or 480 mg (**5**, **6**, **16**, **17**, **20**, **23**) of a 1.6% solution of KOH in the alcohol. The mixture was neutralized with solid CO_2 while the reaction vessel was immersed into a water bath at room temperature. The product was separated from excess alcohol and byproducts by column chromatography on silica gel (neutral alumina for **9**) and/or preparative TLC on silica gel (neutral Al_2O_3 for **26**) using the solvent systems given Table III. In the purification of **25**, the excess alcohol was removed by rotary evaporation and the residue was treated with ether to give a brownish solid that was crystallized from ether and the least amount of acetone. Two preparations required additional KOH solution: 780 mg was used for **4** and 710 mg was used for **13**. The preparation of **10** involved a variation of the usual method. In this variation, a solution of *N*-methylmitomycin A (60 mg, 0.17 mmol) in 8 mL of dry tetrahydrofuran was treated with 5 drops of a 1.2% of KOH in ethylene glycol. After 1 h the solvent was removed by rotary evaporation and the residue was purified by TLC on silica gel with $CHCl_3$–MeOH (8:2) as solvent. The spot containing product was extracted into CH_2Cl_2, which was filtered and concentrated. Yields and physical properties for the products of method B are given in Table I.

Method C. A solution of 7-hydroxymitosane (obtained from the hydrolysis of mitomycin C: 95 mg for **9**, 236 mg for 11, 500 mg for **19**, 300 mg for **21**, and 100 mg for **24**) in CH_2Cl_2 (30–70 mL) was stirred with a solution of excess (1.5–10-fold) 3-substituted 1-phenyltriazene in a few milliliters of CH_2Cl_2 for 48–96 h (monitored by TLC). The solvent was removed under reduced pressure and the residue was purified by preparative TLC on silica gel with $CHCl_3$–MeOH (9:1) or $CHCl_3$–acetone (1:1) as the solvent. Compound **19** required further TLC on neutral alumina with $CHCl_3$–acetone (1:1) as the solvent.

A modified procedure was used for **12**. In this modification, the 7-hydroxymitosane (obtained from 200 mg of mitomycin C) was dissolved in 200 mL of ether, a few milliliters of CH_2Cl_2 was added to clarify the solution, and it was stirred for 40 h. The solid that formed was collected and washed well with ether.

The yields and physical properdties of compounds prepared by method C are given in Table I. Preparations of the triazenes are described below.

Method D. A solution of 7-hydroxymitosane (100 mg, 0.286 mmol) in 25 mL of ethyl acetate was stirred with dicyclohexylcarbodiimide (124 mg, 0.6 mmol) under N_2 for 12 h. The mixture was washed with 0.2 N sodium hydroxide and water, dried over $MgSO_4$, and concentrated under reduced pressure. The product **27** was purified by preparative TLC on silica gel with $CHCl_3$–MeOH (8:2) as solvent. Its yield and physical properties are given in Table I.

Preparation of 3-Substituted 1-Phenyltriazenes. A cold (0 °C) solution of 1 equiv of the appropriate amine free base in *N*,*N*-dimethylformamide containing excess K_2CO_3 was stirred and treated with a cold solution of 1.1 equiv of benzenediazonium hexafluorophosphate in *N*,*N*-dimethylformamide, added in portions. In two preparations the amount of diazonium salt was increased: 2 equiv for the 2-hydroxyethyl derivative and 1.5 equiv for the furfuryl derivative. After the addition was complete, the mixture was stirred 2 h at 0–5 °C and then poured into ice water. The furfuryl derivative solidified and was collected. Other products were taken into ether, washed with water, dried, and concentrated under reduced pressure. The residues were utilized as described below.

3-(2-Hydroxyethyl)-1-phenyltriazene was obtained in 81% yield as a yellow oil that was used without further purification: ^1H NMR ($CDCl_3$) δ 3.65 (s, 4, $2CH_2$), 7–7.6 (m, 5, phenyl).

3-(3-Hydroxypropyl)-1-phenyltriazene was obtained in 30% yield as a yellow oil. It crystallized from hexane as pale yellow needles: mp 56–58 °C; ^1H NMR ($CDCl_3$) δ 1.55–2.2 (m, 2, CCH_2C), 2.25–3.0 (br s, 1, OH), 3.45–4.0 (m, 4, CH_2N and CH_2O), 6.95–7.45 (m, 5, phenyl), 8.05–9.05 (br, 1, NH). Anal. ($C_9H_{13}N_3O$) C, H; N: calcd, 23.46; found, 22.16.

3-(2,3-Dihydroxypropyl)-1-phenyltriazene was extracted five times with boiling hexane and the residual solid was crystallized from $CHCl_3$. This procedure gave 13% of pale yellow needles: mp 97–98 °C; ^1H NMR ($CDCl_3$) δ 3.2–4.2 (m, 7, CH_2OH + $CHOH$ + CH_2N), 6.85–7.45 (m, 5, phenyl), 7.65–8.1 (br, 1, NH). Anal. ($C_9H_{13}N_3O_2$) C, H, N.

3-Furfuryl-1-phenyltriazene was obtained as a solid in 53% crude yield. Recrystallization from hexane gave pale yellow needles: mp 68–70 °C; ^1H NMR ($CDCl_3$) δ 4.7–4.9 (d, 2, CH_2N, 6.2–6.45 (t, 2, furan positions 3 and 4), 7.0–7.55 (m, 6, phenyl and furan position 5), 8.3–9.0 (br, 1, NH). Anal. ($C_{11}H_{11}N_3O$) C, H, N.

3-(2,2-Dimethoxyethyl)-1-phenyltriazene was obtained in 48% yield as a red oil that was used without further purification: ^1H NMR (petroleum ether) δ 3.3 (s, 6, $2CH_3O$), 3.7–3.9 (d, 2, CH_2N), 4.5–4.8 (t, 1, OCHO), 7.0–7.6 (m, 5, phenyl), 8.45–9.15 (br, 1, NH).

3-(2-Cyanoethyl)-1-phenyltriazene was crystallized from hexane to give a 28% yield of pale yellow needles: mp 70–71 °C; ^1H NMR ($CDCl_3$) δ 2.65–3.2 (t, 2, CH_2CN), 3.8–4.4 (t, 2, CH_2NH), 7.0–7.9 (m, 5, phenyl), 8.6–9.3 (br, 1, NH). Anal. ($C_9H_{10}N_4$) C, H, N.

The yields of the furfuryl- and 2-hydroxyethyl-substituted triazenes were improved by increasing the ratio of diazonium salt to amine. In the former compound, the yield went from 12% to 53% when the ratio was changed from 1.0 to 1.5. Although a 2:1 ratio gave a good yield of the latter compound, increasing the ratio to 5:1 gave an unexpected result. The only product isolated was a small amount (2%) of **1,5-diphenyl-3-(2-hydroxyethyl)pentazene**, which had mp 130–131 °C after crystallization from hexane: ^1H NMR ($CDCl_3$) δ 2.8–3.1 (t, 1, OH), 3.8–4.2 (q, 2, CH_2O), 4.8–5.1 (t, 2, CH_2N), 7.4–8.0 (m, 10, phenyls). Anal. ($C_{14}H_{15}N_5O$) C, H, N.

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Registry No. 1, 4055-39-4; **2**, 56981-59-0; **3**, 56981-61-4; **4**,

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105139-94-4; **5**, 105139-95-5; **6**, 105139-96-6; **7**, 56981-68-1; **8**, 105139-97-7; **9**, 105139-98-8; **10**, 105162-44-5; **11**, 105139-99-9; **12**, 105162-45-6; **13**, 105140-00-9; **14**, 105140-01-0; **15**, 105140-02-1; **16**, 105140-03-2; **17**, 105140-04-3; **18**, 105140-05-4; **19**, 105140-06-5; **20**, 105140-07-6; **21**, 105140-08-7; **22**, 105140-09-8; **23**, 105140-10-1; **24**, 105140-11-2; **25**, 105140-12-3; **26**, 103864-79-5; **27**, 105140-13-4; N_2CH_2, 334-88-3; N_2CHMe, 1117-96-0; N_2CHPh, 766-91-6; $HO(CH_2)_2OH$, 107-21-1; *i*-PrOH, 67-63-0; $CH_2{=}CHCH_2OH$, 107-18-6; $CH{\equiv}CCH_2OH$, 107-19-7; $PhCH_2OH$, 100-51-6; $Cl(CH_2)_3OH$, 627-30-5; $HO(CH_2)_3OH$, 504-63-2; $HO(CH_2)_2O(CH_2)_2OH$, 111-46-6; $HO(CH_2)_2S(CH_2)_2OH$, 111-48-8; $HO(CH_2)_2SS(CH_2)_2OH$, 1892-29-1; MeO, 111-77-3; $EtO(CH_2)_2O(CH_2)_2OH$, 111-90-0; $Me_2N(CH_2)_2OH$, 108-01-0; $PhN{=}NNH(CH_2)_2OH$, 105140-14-5; $PhN{=}NNH(CH_2)_3OH$, 105140-15-6; $NH_2(CH_2)_3OH$, 156-87-6; $PhN{=}NNHCH_2CH(OH)CH_2OH$, 105140-16-7; $NH_2CH_2CH(OH)CH_2OH$, 616-30-8; $PhN{=}NNHCH_iCH(OMe)_2$, 105140-18-9; $PhN{=}NNH(CH_2)_2CN$, 105140-19-0; $NH_2CH_2CH(OMe)_2$, 22483-09-6; $NH_2(CH_2)_2CN$, 151-18-8; $NH_2(CH_2)_2OH$, 141-43-5; mitomycin C, 50-07-7; 7-hydroxymitosane, 7041-61-4; *N*-methylmitomycin A, 18209-14-8; cyclobutylmethanol, 4415-82-1; tetrahydrofurfurol, 97-99-4; 3-tetrahydrofuranol, 453-20-3; tetrahydropyranmethanol, 100-72-1; 2,2-dimethyl-4-(hydroxymethyl)dioxolane, 98-00-0; 4-morpholineethanol, 622-40-2; dicyclohexylcarbodiimide, 538-75-0; benzenediazonium hexafluorophosphate, 369-58-4; 3-furfuryl-1-phenyltriazene, 105140-17-8; furfurylamine, 617-89-0.

Substituted (Aryloxy)alkanoic Acids as Antagonists of Slow-Reacting Substance of Anaphylaxis

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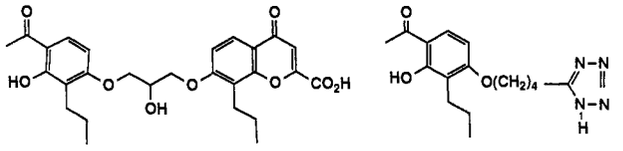
A series of compounds in which the 4-acetyl-3-hydroxy-2-propylphenoxy moiety of the standard SRS-A antagonist, FPL-55712, is linked by a polymethylene or a polyether chain to substituted (aryloxy)alkanoic acids was prepared. The compounds were evaluated for their ability to antagonize SRS-A-induced contractions of guinea pig ilea and LTE-induced bronchoconstriction in the guinea pig. The results showed that the compounds were all less potent than FPL-55712 in vitro, yet surprisingly, most were more potent by the inhalation route of administration. Some of the most potent analogues were selected for further pharmacological evaluation and, by inhalation, exhibited selective antagonism of leukotrienes as compared with PAF or histamine. In comparison to FPL-55712, compounds **28** and **37** were more potent against LTE (40- and 80-fold, respectively), LTD (4- and 3-fold, respectively), and LTC (27- and 20-fold, respectively) induced bronchoconstriction when tested by inhalation.

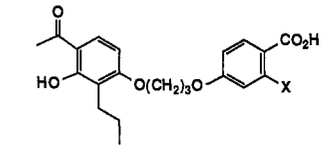
In recent years, evidence has accumulated to support the concept that the bronchoactive peptidoleukotrienes, leukotriene C_4, D_4, and E_4 (LTC, LTD, and LTE), which constitute slow-reacting substance of anaphylaxis (SRS-A) are the predominant mediators of immediate hypersensitivity reactions.[1,2] For this reason, there has been considerable interest in the pharmaceutical industry in the development of leukotriene receptor antagonists as potential therapeutics for the treatment of allergic asthma and other diseases whose pathophysiology may be mediated by leukotrienes.

FPL-55712 (**1**)[3] has long been used as a pharmacological tool because it is a potent SRS-A antagonist, yet it is considered to have limited clinical potential because it is poorly absorbed when given orally and displays a short biological half-life after intravenous administration.[4] Many analogues of **1** have been described.[2] However, there was no evidence that a drug of this structural type was under development until recently when a tetrazole, LY171883 (**2**),[5] was reported to be an orally active LTD antagonist.

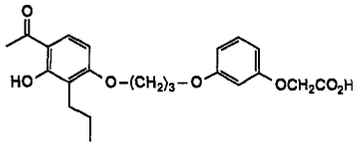
We previously observed that aerosol administration of **1** effectively protected guinea pigs against leukotriene-induced bronchoconstriction with a reasonably long biological half-life.[6] Other investigators showed that aerosolized **1** abolished the cough response and bronchoconstriction due to inhalation of LTC and LTD in two normal volunteers[7] and significantly improved forced expiratory volume in two of four chronic asthmatic patients.[8] Taken together, these results with inhaled **1** prompted us to investigate the development of an aerosolized SRS-A antagonist. Inhalation may be the preferred route of ad-



FPL-55712 (1) LY 171883 (2)



3, (X = H)
4, (X = OH)



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