Antiestrogens. Synthesis and Evaluation of Mammary Tumor Inhibiting Activity of 1,1,2,2-Tetraalkyl-1,2-diphenylethanes

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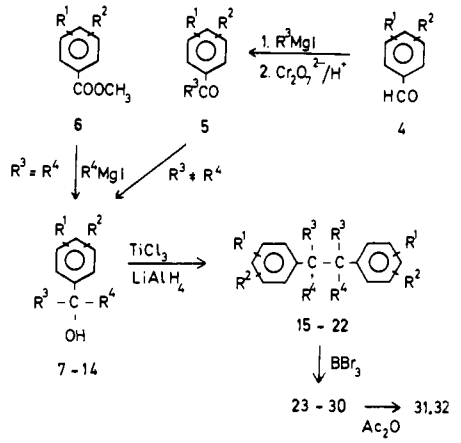
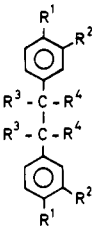
Among the newly synthesized 1,1,2,2-tetraalkyl-1,2-diphenylethanes, 1,1,2,2-tetramethyl-1,2-bis(4'-hydroxyphenyl)ethane (**23**) and 1,1,2,2-tetramethyl-1,2-bis(3'-hydroxyphenyl)ethane (**26**) were the most active compounds regarding estradiol receptor affinity, exhibiting K_a values of 0.73×10^8 and 0.67×10^8 M^{-1}, respectively. In vivo, **23** and **26** showed only very small uterotrophic activity in the mouse. They strongly inhibited (73%) the estrone-stimulated mouse uterine growth. Tested on the 9,10-dimethyl-1,2-benzanthracene induced hormone-dependent mammary adenocarcinoma of the Sprague–Dawley rat, compounds **23** and **26** exhibited a dose-dependent inhibition of the tumor growth, having a strong effect at a dose of 20 (mg/kg)/day (compound **23**).

In premenopausal women bearing estrogen receptor positive mammary carcinoma with metastases, ovariectomy is the therapeutic method of choice.[1,2] The tumor-inhibiting effect of ovariectomy is decreased by nonovarial estrogens.[2,3] It is described that the adrenal glands secrete the androgen androstenedione, which is converted into the estrogenic hormone estrone in peripheral tissues.[4-6] This has been the rationale for surgical adrenalectomy.[4] In this regard, it is of interest that some drugs used to treat breast cancer, such as glucocorticoids,[4] aminoglutethimide,[7-13] and Δ^1-testolactone,[5] act by reducing peripheral estrogen production.[5] The additional application of such agents could increase the therapeutic effect of ovariectomy. Tumor growth stimulating effects of nonovarial estrogens could also be eliminated by aftertreatment of ovariectomized patients bearing disseminated mammary carcinoma with antiestrogens. Fiebig and Schmähl,[14] who studied the combination therapy of ovariectomy and tamoxifen application on the DMBA-induced hormone-dependent mammary carcinoma of the SD rat, found a significantly less inhibition of the tumor in comparison to the ovariectomized control. Corresponding results were achieved with the combination ovariectomy and nafoxidin on the hormone-dependent mammary carcinoma of the rat.[15]

Scheme I



Tamoxifen and nafoxidin are partial antiestrogens. Due to their estrogenic side effects they give rise to a more or less pronounced stimulation of the tumor growth at low endogenous estrogen levels (like in ovariectomy), depending on the applied doses. Experiments to find antiestrogens without estrogenic side effects by chemical variation of the synthetic estrogen hexestrol led to the new compounds **1–3**.[16] These compounds are partial anti-



1, $R^1, R^4 = H$; $R^2 = OH$; $R^3 = Et$

2, $R^1, R^2 = OH$; $R^3 = Et$; $R^4 = H$

3, $R^1 = F$; $R^2 = OH$; $R^3 = Et$; $R^4 = H$

estrogens with marked inhibitory activity on the hormone-dependent mammary carcinoma of the rat. Tetraalkylation in the 1,2 position of the diphenylethane skeleton of hexestrol and of compounds **1–3** was performed to achieve a further decrease or loss of estrogenic activity and an increase of antiestrogenic and tumor-inhibiting properties. In the following paragraphs, the synthesis, endocrinological studies, and testing on the DMBA-induced hormone-dependent mammary carcinoma of the rat

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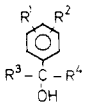
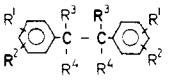
Table I. Substituted Dialkylbenzyl Alcohols



no.	R^1	R^2	R^3	R^4	synth method[a]	yield,[b] %	mp or bp, °C	recrystn solvent[c]	formula[d]
7[f]	4-OCH_3	H	CH_3	CH_3	A	74	101 (0.5)[e]		$C_{10}H_{14}O_2$
8[g]	4-OCH_3	H	CH_3	C_2H_5	A	79	104 (0.5)[e]		$C_{11}H_{16}O_2$
9[h]	4-OCH_3	H	C_2H_5	C_2H_5	A	70	105 (0.5)[e]		$C_{12}H_{18}O_2$
10[i]	3-OCH_3	H	CH_3	CH_3	A	70	34	E	$C_{10}H_{14}O_2$
11	3-OCH_3	H	C_2H_5	C_2H_5	A	79	110 (0.5)[e]		$C_{12}H_{18}O_2$
12[j]	3-OCH_3	4-OCH_3	CH_3	CH_3	A	65	76	F	$C_{11}H_{16}O_3$
13[k]	3-OCH_3	5-OCH_3	CH_3	CH_3	A	86	55	F	$C_{11}H_{16}O_3$
14	3-OCH_3	4-F	CH_3	CH_3	A	89	106 (0.5)[e]		$C_{10}H_{13}FO_2$

[a] Capital letters refer to synthetic methods A–D under Experimental Section. [b] Yield of analytically pure product; no effort was made to optimize yields. [c] E = toluene–ligroin; F = ligroin. [d] All compounds were analyzed for C and H within ±0.40% of the calculated values. [e] Boiling point (mmHg). [f] See ref 18. [g] See ref 19. [h] See ref 20. [i] See ref 21. [j] See ref 22. [k] See ref 23.

Table II. Substituted Tetraalkylbibenzyls



no.	R^1	R^2	R^3	R^4	synth method[a]	yield,[b] %	mp, °C	recrystn solvent[c]	formula[d]
15[e]	4-OCH_3	H	CH_3	CH_3	B	60	184	E	$C_{20}H_{26}O_2$
16[f]	4-OCH_3	H	CH_3	C_2H_5	B	65	90–94	G	$C_{22}H_{30}O_2$
17	4-OCH_3	H	C_2H_5	C_2H_5	B	73	71–72	G	$C_{24}H_{34}O_2$
18	3-OCH_3	H	CH_3	CH_3	B	80	116	E	$C_{20}H_{26}O_2$
19	3-OCH_3	H	C_2H_5	C_2H_5	B	70	oil		$C_{24}H_{34}O_2$
20	3-OCH_3	4-OCH_3	CH_3	CH_3	B	65	104–106	E	$C_{22}H_{30}O_4$
21	3-OCH_3	5-OCH_3	CH_3	CH_3	B	64	118	E	$C_{22}H_{30}O_4$
22	3-OCH_3	4-F	CH_3	CH_3	B	89	126–128	E	$C_{20}H_{24}F_2O_2$
23[e]	4-OH	H	CH_3	CH_3	C	89	210	H	$C_{18}H_{22}O_2$
24[f,g]	4-OH	H	CH_3	C_2H_5	C	76	170–174	H	$C_{20}H_{26}O_2$
25[g]	4-OH	H	C_2H_5	C_2H_5	C	78	105	H	$C_{22}H_{30}O_2$
26	3-OH	H	CH_3	CH_3	C	91	200	H	$C_{18}H_{22}O_2$
27	3-OH	H	C_2H_5	C_2H_5	C	80	174	H	$C_{22}H_{30}O_2$
28	3-OH	4-OH	CH_3	CH_3	C[h]	91	190–193	I	$C_{18}H_{22}O_4$
29	3-OH	5-OH	CH_3	CH_3	C[h]	90	270–273	I	$C_{18}H_{22}O_4$
30	3-OH	4-F	CH_3	CH_3	C	67	169–170	H	$C_{18}H_{20}F_2O_2$
31	3-$OCOCH_3$	4-$OCOCH_3$	CH_3	CH_3	D	94	225–227	G	$C_{26}H_{30}O_8$
32	3-$OCOCH_3$	5-$OCOCH_3$	CH_3	CH_3	D	95	125–127	G	$C_{26}H_{30}O_8$

[a] Capital letters refer to synthetic methods A–D under Experimental Section. [b] Yield of analytically pure product; no effort was made to optimize yields. [c] E = toluene–ligroin; G = EtOH; H = 80% acetic acid; I = dioxane–H_2O. [d] All compounds were analyzed for C and H within ±0.40% of the calculated values. [e] See ref 24. [f] Meso form. [g] See ref 25. [h] Product was not extracted with NaOH solution.

of 1,1,2,2-tetraalkyl-1,2-diphenylethanes will be described.

Chemistry. In order to synthesize bibenzyl derivatives with identical alkyl substituents ($R^3 = R^4 = CH_3$, C_2H_5, respectively), the substituted methyl benzoates (**6**) were converted with alkylmagnesium iodide into the tertiary alcohols **7** and **9–14** (Scheme I; Table I). The bibenzyl derivatives **15** and **17–22** were prepared by reductive coupling of **7** and **9–14** using $TiCl_3$–$LiAlH_4$ according to the method of McMurry and Silvestri[17] (Table II). The cleavage of the ethers **15** and **17–22** was accomplished with BBr_3 (Table II). Acetylation of the tetrahydroxy derivatives **28** and **29** with acetic anhydride gave **31** and **32** (Table II).

In order to synthesize the bibenzyl derivative **24** with different alkyl substituents ($R^3 = CH_3$; $R^4 = C_2H_5$), 4-methoxybenzaldehyde (**4**) was converted with methylmagnesium iodide to 1-(4′-methoxyphenyl)ethanol. The latter was oxidized with $Na_2Cr_2O_7/H_2SO_4$ to yield the ketone **5**. Compound **8** was prepared by reaction of **5** with ethylmagnesium iodide (Table I). The reductive coupling of **8** using $TiCl_3$–$LiAlH_4$ gave a mixture of *meso*- and *d,l*-3,4-bis(4′-methoxyphenyl)-3,4-dimethylhexane. From this, one diastereoisomer (compound **16**) was separated by crystallization from EtOH (Table II). Compound **16** was converted to the corresponding hydroxy derivative **24** by ether cleavage with BBr_3 (Table II). The latter compound was found to be identical with *meso*-3,4-bis(4′-hydroxyphenyl)-3,4-dimethylhexane, described by Huang and Lee.[25]

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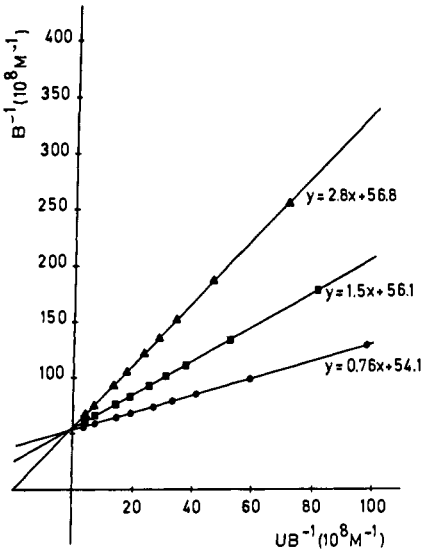

Figure 1. Competitive inhibition of the estradiol receptor interaction by compound **26**: (●–●) [^{3}H]estradiol ($K_a = 0.72 \times 10^{10}$ M^{-1}); (■–■) [^{3}H]estradiol and **26** (10^{-8} M) ($K_a = 0.9 \times 10^8$ M^{-1}); (▲–▲) [^{3}H]estradiol and **26** (2.5×10^{-8} M) ($K_a = 1.0 \times 10^8$ M^{-1}).

Biological Properties. In vitro, 1,1,2,2-tetraalkyl-1,2-diphenylethanes inhibited the estradiol receptor interaction competitively (Figure 1).

The 1,1,2,2-tetramethyl-1,2-diphenylethanes **23**, **26**, **28**, and **30** exhibited a smaller binding affinity for the estradiol receptor than the corresponding 3,4-diphenylhexanes hexestrol and **1**–**3**. Hexestrol, **1**, and **2** had similar association constants compared to each other, whereas **3** was less active[16] (K_a values of hexestrol: 0.98×10^9 M^{-1}; **1**: 0.35×10^9 M^{-1}; **2**: 0.86×10^9 M^{-1}; **3**: 0.65×10^8 M^{-1}). Among the tested 1,1,2,2-tetraalkyl-1,2-diphenylethanes, compounds **23** and **26** were the most active ones. They had K_a values of 0.73×10^8 and 0.67×10^8 M^{-1}, respectively (Table III).

The association constants of 1,2-dimethyl-1,2-diethyl-1,2-diphenylethane (**24**) and the 1,1,2,2-tetraethyl-1,2-diphenylethanes **25** and **27** were smaller compared to the tetramethyl derivatives. The change from the catechol structure (**28**) to the 3,5-dihydroxy configuration (**29**), as well as the transformation of **28** to the acetylated product **31**, lessened the binding affinity markedly. The acetylation of **29** did not change receptor affinity (see **32**).

Similar to the results of the in vitro experiment was the gradation of the antiestrogenic activity of the 1,1,2,2-tetraalkyl-1,2-diphenylethanes in the mouse uterine weight test in vivo (Table IV). In this experiment too, the compounds **23** and **26**—derived from the estrogen hexestrol and the partial antiestrogen **1**—were the most active ones. They exhibited 73% inhibition of the estrone-stimulated uterine growth. It is important that these strongly antiuterotrophic agents had only very small uterotrophic activity (12 and 15% of the maximum estrone activity), whereas the partial antiestrogenic hexestrol derivatives **1**–**3** reached in high dosage about 65% of the estrone activity.[16] The uterotrophic activity of **2** and **3** was also markedly reduced by tetramethylation of the diphenylethane skeleton of **2** and **3** (compounds **28** and **30**). The antiuterotrophic activity was somewhat decreased as it could be expected from the in vitro test. Compounds **29**, **31**, and **32**, which had the least affinity for the estradiol receptor, exhibited very low uterotrophic and no antiuterotrophic effects. The 1,2-dimethyl-1,2-diethyl-1,2-diphenylethane **24** and the 1,1,2,2-tetraethyl-1,2-diphenylethanes **25** and **27** reached in high doses the maximum uterotrophic activity of estrone. Small doses of **24**, **25**, and **27** had estrone-antagonizing activity, whereas higher doses had none. This is typical of impeded estrogens.

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Table III. Relative Activity (RA), Ratio of Association Constants (RAC), and Association Constants (K_a) of **1** and **23**–**32**

no.	RA[a]	RAC[b]	K_a,[c] M^{-1}
1	5.2×10^{-2}	4.8×10^{-2}	0.35×10^9
23	1.1×10^{-2}	1.0×10^{-2}	0.73×10^8
24	2.7×10^{-3}	2.5×10^{-3}	0.18×10^8
25	5.0×10^{-3}	4.6×10^{-3}	0.48×10^8
26	1.0×10^{-2}	9.3×10^{-3}	0.67×10^8
27	4.2×10^{-3}	3.9×10^{-3}	0.28×10^8
28	6.0×10^{-3}	5.6×10^{-3}	0.40×10^8
29	4.3×10^{-5}	4.0×10^{-5}	0.29×10^6
30	3.1×10^{-3}	2.9×10^{-3}	0.21×10^8
31	2.3×10^{-4}	2.1×10^{-4}	0.15×10^7
32	7.1×10^{-5}	6.6×10^{-5}	0.48×10^6

[a] RA = E_2/I, E_2 and I are the molar concentrations of unradioactive E_2 and inhibitor substance required to halve the bound radioactivity; E_2 = 17β-estradiol. [b] RAC = $[R(RA)]/[R + 1 - RA]$, R = free/bound radioactivity. [c] $K_a = K_a(E_2) \times$ RAC. The association constant of estradiol was used as determined in Figure 1 ($K_a(E_2) = 0.72 \times 10^{10}$).

The most active inhibitors of estradiol receptor interaction (**23**, **26**, and **28**) were tested on the DMBA-induced hormone-dependent mammary carcinoma of the rat. Tested in the same concentrations as the potent mammary tumor growth inhibiting substances **1**–**3**,[16] the dose-dependent activity of the 1,1,2,2-tetramethyl-1,2-diphenylethanes was found to be markedly decreased (percent change of tumor area in control: 573%. **23**: 0.5 mg, 607%; 1 mg, 411%; 4 mg, 275%. **26**: 0.5 mg, 562%; 1 mg, 445%; 4 mg, 375%. **28**: 0.5 mg, 604%; 1 mg, 557%; 4 mg, 320%. Tamoxifen: 3 mg, 95%. Ovariectomy: −7%; see also Figure 2).

Because of the smaller association constants of the 1,1,2,2-tetramethyl-1,2-diphenylethanes compared to **1**, compounds **23** and **26** were tested in a dose of 20 mg/kg, five times as much as the most active tumor-inhibiting dose of **1** (4 mg/kg)[16] (Figure 3). This high dosage led to a strong inhibition of the tumor growth. Compound **23** was almost as active as **1**.[16] Compound **26** was less active but still inhibited the tumor growth markedly (percent change of tumor area in control: 554%. **23**: 20 mg, 11%. **26**: 20 mg, 168%. Ovariectomy: −90%).

Consistent with the observed very low estrogenic activity (**23** and **26**) in the mouse uterus, the uterine weights of the tumor-bearing rats treated with **23**, **26**, and **28** were not significantly different from the weights of the control group, whereas tamoxifen led to a significant decrease of uterine weight at the end of the experiment (**23**: 0.5 mg, −21%; 1 mg, −10%; 4 mg, −19%; 20 mg, +8%. **26**: 0.5 mg, −20%; 1 mg, −13%; 4 mg, −17%; 20 mg, −17%. **28**: 0.5 mg, −13%; 1 mg, −2%; 4 mg, −15%. Tamixofen: 3 mg, −37%. The U-test according to Wilcoxon et al. was used, $\alpha = 0.05$. Mammary tumor inhibiting estrogens, such as diethylstilbestrol, increase uterine weight). The weights of the ovaries and the adrenal glands were also not significantly different from the weights of the control group.

Based on these results, it becomes apparent that one can get antiestrogens not only by displacement of the phenolic OH groups of hexestrol into the 3,3′ position (compound **1**) but also by tetramethylation in the 1,2 position of the diphenylethane skeleton of hexestrol. The last procedure leads virtually to a loss of uterotrophic activity while the

Table IV. Estrogenic and Antiestrogenic Activity of Compounds 23-32 in Mouse Uterine Weight Test

	uterotrophic test		antiuterotrophic test		
compd	dose,[a] μg	effect[b,d]	dose,[a] μg	effect[b]	% inhibn[c,d]
23	0	9.2 ± 2.7	0	11.2 ± 2.1	
	8	10.6 ± 2.0[e]	5	37.3 ± 2.1	15.4[f]
	24	15.7 ± 1.7[g]	25	28.0 ± 3.2	45.4[h]
	80	15.8 ± 2.8[g]	100	19.4 ± 4.0	73.5[h]
	250	13.3 ± 2.0[g]	250	19.3 ± 5.0	73.7[h]
	1000	14.3 ± 2.3[g]	1000	24.4 ± 4.5	57.3[h]
estrone	0.4	45.3 ± 4.7	0.1	42.0 ± 5.0	
24	0	15.4 ± 2.1	0	14.0 ± 2.1	
	2	24.6 ± 6.3[g]	10	34.6 ± 3.8	10.4[f]
	10	34.7 ± 3.8[h]	25	34.0 ± 5.1	13.1[e]
	50	39.1 ± 4.8[h]	100	38.2 ± 1.5	
	250	50.1 ± 7.2[h]	500	42.2 ± 3.3	
estrone	0.4	54.3 ± 6.5	0.1	36.9 ± 1.4	
25	0	9.2 ± 2.7	0	14.0 ± 2.1	
	8	15.3 ± 2.2[g]	2	31.3 ± 2.1	24.6[g]
	24	23.6 ± 2.6[h]	10	34.6 ± 1.1	10.3[e]
	80	31.9 ± 4.9[h]	25	36.4 ± 1.6	2.2[e]
	240	43.7 ± 5.1[h]	250	43.2 ± 6.9	
estrone	0.4	45.3 ± 4.7	0.1	37.0 ± 1.4	
26	0	9.2 ± 2.7	0	11.2 ± 2.1	
	8	9.8 ± 2.0[e]	5	33.9 ± 4.0	26.5[h]
	24	15.7 ± 2.2[g]	25	28.7 ± 4.7	43.2[h]
	80	15.4 ± 4.7[g]	100	20.4 ± 4.7	70.3[h]
	250	14.6 ± 2.2[g]	250	19.4 ± 3.4	73.6[h]
	1000	13.4 ± 1.7[g]	1000	23.4 ± 2.9	60.5[h]
estrone	0.4	45.3 ± 4.7	0.1	42.0 ± 5.0	
27	0	15.4 ± 2.1	0	15.6 ± 2.3	
	4	23.1 ± 4.4[h]	2	27.4 ± 1.7	47.0[h]
	20	35.8 ± 5.9[h]	10	32.8 ± 4.4	23.0[g]
	100	52.7 ± 5.6[h]	25	38.0 ± 4.6	
	500	52.4 ± 5.2[h]	250	42.9 ± 7.8	
estrone	0.4	54.3 ± 6.5	0.1	37.9 ± 2.2	
28	0	9.6 ± 2.9	0	15.7 ± 1.3	
	5	9.7 ± 3.3[e]	5	38.6 ± 5.4	6.8[e]
	25	8.9 ± 2.0[e]	25	35.0 ± 2.5	21.5[g]
	100	10.0 ± 1.9[e]	50	32.8 ± 1.5	30.4[h]
	500	12.5 ± 3.1[e]	100	28.4 ± 4.5	45.5[h]
estrone	0.4	39.6 ± 3.2	0.1	40.3 ± 5.1	
29	0	9.6 ± 2.9	0	11.3 ± 3.7	
	5	9.0 ± 3.1[e]	10	30.6 ± 3.6	11.7[e]
	25	9.4 ± 2.9[e]	100	40.4 ± 6.1	
	100	8.5 ± 1.8[f]	500	38.9 ± 6.4	
	500	14.3 ± 3.3[f]			
estrone	0.4	39.6 ± 3.2	0.1	33.1 ± 5.2	
30	0	15.4 ± 2.1	0	15.7 ± 1.3	
	4	17.8 ± 4.0[f]	5	36.1 ± 3.5	17.2[f]
	20	16.7 ± 2.3[e]	25	35.2 ± 3.5	20.9[g]
	100	16.7 ± 4.5[e]	100	27.3 ± 2.7	53.1[h]
	500	21.4 ± 5.4[g]	500	25.2 ± 2.6	61.5[h]
estrone	0.4	54.3 ± 6.5	0.1	40.3 ± 5.1	
31	0	11.4 ± 1.8	0	12.6 ± 2.1	
	2.5	13.8 ± 2.2[f]	2.5	36.4 ± 6.3	18.3[e]
	10	13.8 ± 2.6[f]	10	37.8 ± 5.2	13.3[e]
	50	13.6 ± 2.2[f]	25	37.8 ± 6.0	13.5[e]
	250	14.2 ± 3.5[f]	100	41.6 ± 7.3	0.3[e]
estrone	0.4	42.6 ± 6.3	250	38.8 ± 5.4	9.4[e]
			0.1	41.7 ± 7.5	
32	0	11.4 ± 1.8	0	12.6 ± 2.1	
	2.5	11.1 ± 1.7[e]	2.5	39.1 ± 5.0	8.9[e]
	10	12.1 ± 2.2[e]	10	37.5 ± 7.1	14.4[e]
	50	14.1 ± 2.1[g]	25	39.0 ± 3.7	9.1[e]
	250	11.3 ± 1.9[e]	100	41.3 ± 3.4	1.6[e]
estrone	0.4	42.6 ± 6.3	250	38.4 ± 5.6	11.4[e]
			0.1	41.7 ± 7.5	

[a] Dose per animal and day. [b] Uterus dry weight (mg)/body weight (g) × 100. [c] Percent inhibition = $100 - (E_{S,T} - E_V)/(E_S - E_V) \times 100$; E_S = effect of estrone standard; $E_{S,T}$ = effect of standard under simultaneous application of test substance; E_V = effect of vehicle. [d] The U test according to Wilcoxon, Mann, and Whitney was used. [e] Not significant ($\alpha = 0.05$). [f] Significant ($\alpha = 0.05$). [g] Significant ($\alpha = 0.01$). [h] Significant ($\alpha = 0.001$).

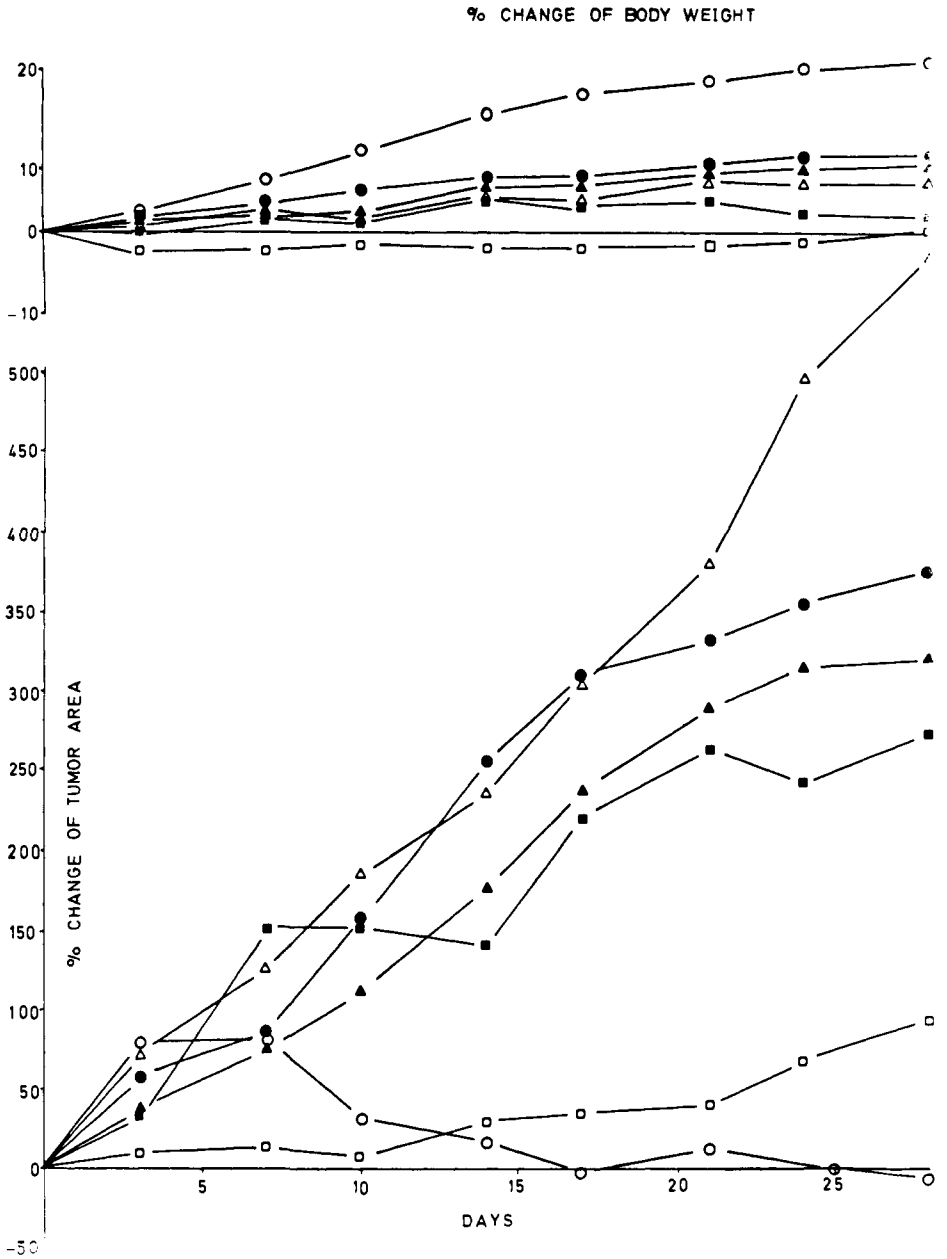

Figure 2. The effect of ovariectomy, tamoxifen, **23**, **26**, and **28** on tumor area and body weight of the SD rat bearing DMBA-induced hormone-dependent mammary tumors: control (△–△), ovariectomy (○–○); tamoxifen, from Monday to Thursday 3 (mg/kg) sc/day, on Friday 6 mg/kg sc (□–□); **23**, from Monday to Thursday 4 (mg/kg) sc/day, on Friday 8 mg/kg sc (■–■); **26**, from Monday to Thursday 4 (mg/kg) sc/day, on Friday 8 mg/kg sc (●–●); **28**, from Monday to Thursday 4 (mg/kg) sc/day, on Friday 8 mg/kg sc (▲–▲).

receptor affinity is retained. For this reason, **23**, **26**, and **28** are potent inhibitors of the estradiol receptor interaction. Just like the antiestrogenic activity generated by displacement of the phenolic OH groups into the 3,3′ position (compound 1) can be destroyed by replacement of the α,β-diethyl groups by isopropyl groups[26] [3,4-bis-(3′-hydroxyphenyl)-2,5-dimethylhexane is a true estrogen], the low uterotrophic activity of 1,1,2,2-tetramethyl-1,2-diphenylethanes (compounds **23** and **26**) is markedly increased by exchange of two or four methyl groups by ethyl groups (compounds **24**, **25**, and **27** are impeded estrogens). Although the hexestrol derivatives **1–3** exhibited much stronger estrogenic side effects than the 1,1,2,2-tetramethyl-1,2-diphenylethanes, they had similar (compared to **23**) or better (compared to **26**) rat mammary tumor inhibiting activity. It is a well-known fact that small doses of estrogens cause stimulation of DMBA-induced mammary adenocarcinoma of ovariectomized rats.[27] Because of their estrogenic side effects, partial antiestrogens, such as the hexestrol derivatives **1–3**, possibly could inhibit the tumor growth stimulated by endogenous estrogens to a smaller extent compared to antiestrogens with no or very small estrogenic side effects. Estrogenic effects of partial antiestrogens reversely could cause inhibition of tumor growth in high dosage, for it is also established that high doses of estrogens inhibit the growth of rat mammary tumors.[28] In the case of the 1,1,2,2-tetramethyl-1,2-diphenylethanes, tumor growth inhibition activity is certainly caused by their antiestrogenic activity, whereas in the case of the partial antiestrogens **1–3** further investigations will be necessary to illuminate to what extent estrogenic effects

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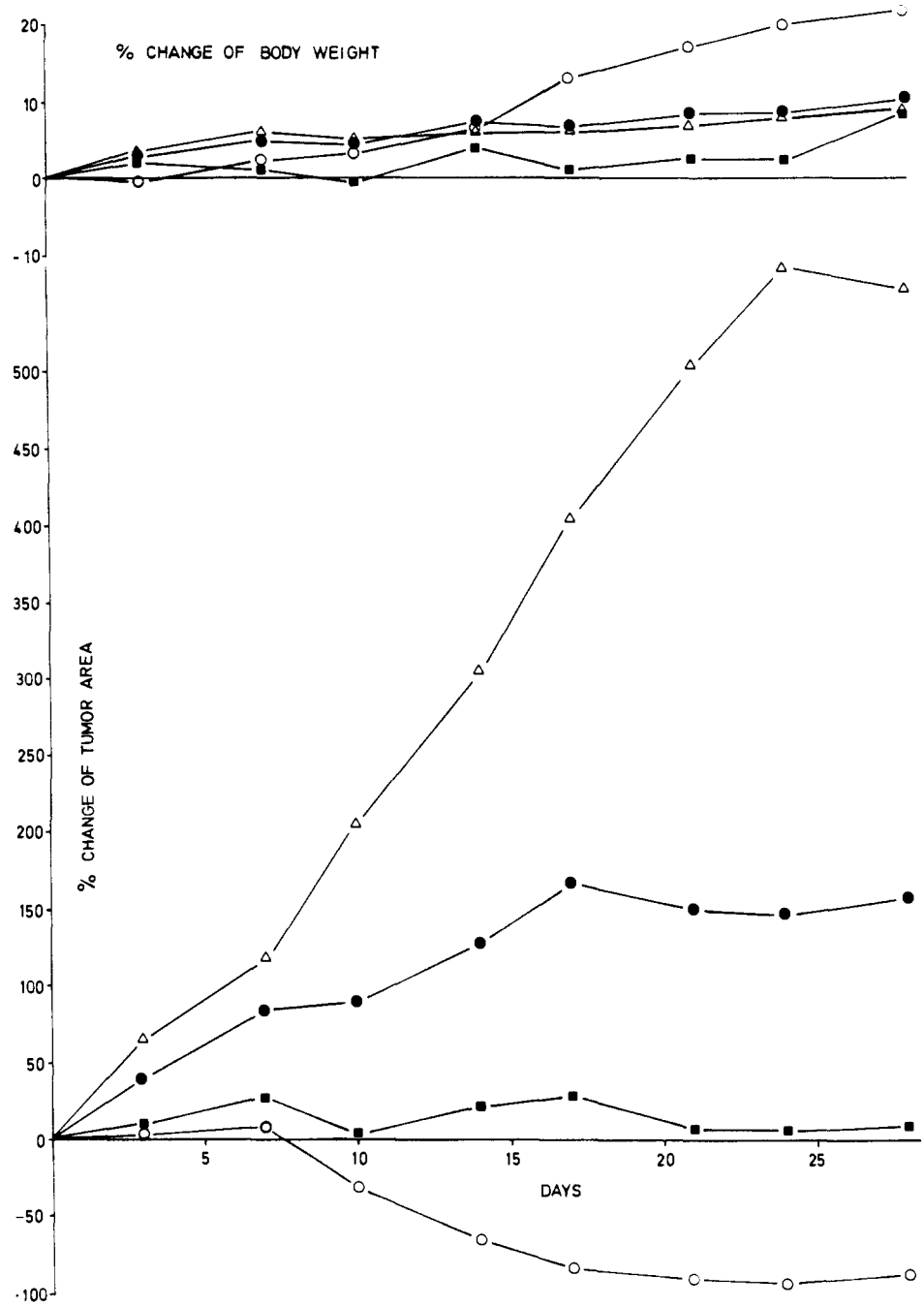

Figure 3. The effect of ovariectomy, **23**, and **26** on tumor area and body weight of the SD rat bearing DMBA-induced hormone-dependent mammary tumors: control (△–△) (CR 3%, PR 3%, NC 28%, P 66%); ovariectomy (○–○) (CR 83%, PR 8%, NC 9%, P 0%); **23**, from Monday to Thursday 20 (mg/kg) sc/day, on Friday 40 mg/kg sc (■–■) (CR 40%, PR 20%, NC 20%, P 20%); **26**, from Monday to Thursday 20 (mg/kg) sc/day, on Friday 40 mg/kg sc (●–●) (CR 11%, PR 28%, NC 19%, P 42%); CR = complete remission, tumor not palpable; PR = partial remission, reduction in tumor size ≥50% of initial tumor size; NC = no change, tumor size 51–150% of initial tumor size; P = progression, tumor size >150% of initial tumor size.

influence tumor growth inhibition activity.

Experimental Section

General Procedures. TLC of each compound was performed on Merck F 254 silica gel plates. Melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. Elemental analyses were performed by the Mikroanalytisches Laboratorium, Universität Regensburg. The structures of all compounds were confirmed by their IR (Beckman AccuLab 3) and ^1H NMR spectra (Varian EM 360A, 60 MHz).

Syntheses. The methyl phenyl ketone **5** was prepared by Grignard reaction of 4-methoxybenzaldehyde in ether[29] and subsequent oxidation with $Na_2Cr_2O_7$ and H_2SO_4 according to the standard method.[30]

Methyl 4-Fluoro-3-methoxybenzoate. A solution of methyl 4-amino-3-methoxybenzoate (36.22 g, 0.2 mol) in 165 mL of 20% tetrafluoroboric acid was cooled to −5 °C. Sodium nitrite (13.80 g, 0.2 mol) dissolved in 25 mL of cold water was added dropwise with stirring. The precipitated diazonium tetrafluoroborate was cooled to −25 °C, collected, washed with cold tetrafluoroboric acid (5%) and water, and dried under vacuum. The diazonium tetrafluoroborate was decomposed by dry distillation under reduced pressure (20 mm) to yield, after recrystallization from EtOH, 17.4

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g (47%) of methyl 4-fluoro-3-methoxybenzoate, mp 40 °C. Anal. ($C_9H_9FO_3$) C, H.

Synthetic methods A–D are representatives for compounds reported in Tables I and II.

Method A. 2-(4′-Fluoro-3′-methoxyphenyl)-2-propanol (14). Methyl iodide (71.0 g, 0.5 mol) was dissolved in ether and added dropwise with stirring to magnesium turnings (12.15 g, 0.5 g-atom) in 50 mL of dry ether. The mixture was heated to reflux for 0.5 h. A solution of methyl 4-fluoro-3-methoxybenzoate (36.83 g, 0.2 mol) in ether was added dropwise with stirring. After heating to reflux for 2 h, the mixture was cooled and poured on ice. The resulting precipitate was dissolved by the addition of a NH_4Cl solution. The ethereal layer was separated and the aqueous layer was extracted with ether. The combined ethereal extracts were washed with solutions of $NaHSO_3$, $NaHCO_3$, and water and dried over anhydrous Na_2SO_4. The solvent was removed and the resulting oil distilled under high vacuum to give 32.8 g of **14**.

Method B. 2,3-Bis(4′-fluoro-3′-methoxyphenyl)-2,3-dimethylbutane (22). Titanium trichloride (23.0 g, 0.15 mol) was weighed under dry nitrogen and placed under nitrogen in a 1-L three-neck flask with 700 mL of dry glyme. $LiAlH_4$ (1.9 g, 0.05 mol) was quickly added to the stirred $TiCl_3$ slurry. The resulting black suspension was stirred for 10 min. Compound **14** (0.21 g, 0.05 mol) was dissolved in 20 mL of dry glyme and added dropwise with stirring. The mixture was heated to reflux and kept there for 16 h. After cooling, the reaction mixture was quenched by the addition of 2 N HCl, then diluted with water, and extracted with ether. The ether extracts were combined, washed with a solution of $NaHCO_3$ and water, and dried over $MgSO_4$. The solvent was removed and the resulting crude product was recrystallized from toluene–ligroin (80:20) to give 7.44 g of **22**.

Method C. 2,3-Bis(4′-fluoro-3′-hydroxyphenyl)-2,3-dimethylbutane (30). A solution of **22** (3.34 g, 0.01 mol) in 250 mL of dry CH_2Cl_2 was cooled to −60 °C. Under nitrogen, BBr_3 (7.52 g, 0.03 mol) was added with stirring. After 0.5 h, the freezing mixture was removed and the reaction mixture was kept at room temperature for 4 h. Fifty milliliters of H_2O was added, and the mixture was shaken with 2 N NaOH. After neutralization of the aqueous layer with 3 N H_2SO_4, the solution was extracted with ether. The ether extracts were washed with $NaHCO_3$ solution and water and dried over anhydrous Na_2SO_4. After removal of the solvent, the crude product was recrystallized from 80% acetic acid to give 2.05 g of **30**.

Method D. 2,3-Bis(3′,4′-diacetoxyphenyl)-2,3-dimethylbutane (31). 2,3-Bis(3′,4′-dihydroxyphenyl)-2,3-dimethylbutane (**28**; 3.02 g, 0.01 mol) was added to a mixture of acetic anhydride (4.08 g, 0.04 mol) and dry pyridine (3.96 g, 0.05 mol). The mixture was heated to reflux for 3 h with stirring. After cooling, the mixture was poured into 300 mL of ice–water and acidified with 1 N HCl. The mixture was extracted with ether. The ether extract was washed with 1 N HCl, $NaHCO_3$ solution, and water and dried over anhydrous Na_2SO_4. The solvent was removed and the crude product recrystallized from EtOH to give 4.42 g of **31**.

Biological Methods. Estradiol Receptor Binding Assay. The technique described in the literature[31-33] was employed with modifications. Cytosol was obtained from a homogenate of calf uteri by centrifugation at 105000*g* in 0.01 M Tris-HCl, 0.001 M EDTA, 0.003 M NaN_3, pH 7.5 buffer. In the relative activity (RA) experiments the following incubation conditions were used: 100 μL aliquots of the cytosol containing 10 mg of protein/mL were mixed with 1 pmol of [^{3}H]estradiol (specific activity 90–115 Ci/nmol) and a competing substance (estradiol, **1**, and **23-32**). The final volume of each mixture was 0.5 mL. Each competitor was run in five concentrations; each concentration was run three times. The incubation was carried out at 4 °C for 16 h. The specific binding of [^{3}H]estradiol to the receptor was assayed by adsorption of the unbound ligand on dextran-coated charcoal. The cytosol was shaken at 4 °C for 90 min with 0.5 mL of a suspension of dextran (0.008%) and charcoal (0.8%) in Tris–EDTA buffer and then centrifuged at 700*g* for 10 min. Aliquots of the supernatant were assayed for specifically bound [^{3}H]estradiol by counting in a toluene scintillation mixture in a Beckman liquid scintillation spectrometer (LS 8000). The efficiency was 42%. The concentration of a competing substance required to halve the bound radioactivity was determined by plotting bound radioactivity vs. log of concentration. In the experiment for the determination of the type of inhibition, 100-μL aliquots of the cytosol were incubated with increasing amounts of [^{3}H]estradiol (0.1, 0.15, 0.2, 0.25, 0.3, 0.4, 0.5, 1, and 2 pmol) and 0, 10, or 25 pmol of compound **26**. The same assay conditions as in the RA experiments were used. The double-reciprocal plot of bound vs. unbound [^{3}H]estradiol according to Lineweaver–Burk[34] (Figure 1) was used to determine the K_a values of **26** (0.95×10^8 M^{-1}, mean value out of two determinations) and estradiol (0.72×10^{10} M^{-1}). The latter was used to calculate the K_a values of **1** and **23–32** according to Korenman[32,35,36] and Ekins.[37] The association constant of **26** determined by calculation (0.67×10^8 M^{-1}) was similar to that determined by the competitive inhibition experiment (0.95×10^8 M^{-1}).

Estrogen and Antiestrogen Assays. The Dorfman uterine weight test was used to determine estrogenic[31,38] and antiestrogenic[31,39] activity. Intact, immature, female NMRI mice (Firma Wiga, Sulzfeld, West Germany) were randomly distributed into groups of 10 animals. Arachis oil solutions containing 0.4 μg of estrone or the corresponding quantity of the test compound per 0.1 mL (uterotrophic test), 0.1 μg of estrone, or a mixture of 0.1 μg of estrone and the corresponding quantity of the test compound per 0.1 mL (antiuterotrophic test) were prepared. The mice were 20 days of age at the start of the assay. They were injected daily for 3 days sc, the injection volumes being 0.1 mL. In both the tests an additional group of mice served as control, receiving only arachis oil injections. The animals were killed 24 h after the last injection, the body weights were determined, and the uteri were removed and fixed with Bouin's solution for 12 h [15 parts of a saturated aqueous solution of picric acid, 5 parts of formaldehyde (40%), and 1 part of acetic acid]. The uteri were dissected free from connective tissue, washed with a saturated alcoholic solution of LiCl, and dried at 100 °C for 24 h. Then the uterine weights were determined.

Mammary Tumor Growth Inhibition Test. The methods used[31] were similar to those described in the literature.[40-42] Female Sprague–Dawley (SD) rats (Zentralinstitut für Versuchstierzucht Hannover, West Germany) were administered a single dose of 20 mg of DMBA (9,10-dimethyl-1,2-benzanthracene) in 1 mL of arachis oil by gastric intubation, when they were 50 days of age. Rats were palpated twice weekly beginning 35 days later. They were assigned to groups of 10 animals when at least one tumor reached an area of 140 mm^2. The tumor area is the product of measurements of two perpendicular diameters, one across the longest dimension. Rats without tumors by day 120 were discarded; their number was inappreciable. Arachis oil solutions of the test compounds were prepared containing 0.5, 1.0, 4.0 (compounds **23**, **26**, and **28**), 3.0 (tamoxifen), and 20.0 (compound **23** and **26**) mg/mL. The animals received a single dose daily from Monday to Thursday, and a double dose on Friday, the injection volumes depending on their body weights. Therapy was continued for 4 weeks. Measurement of tumor size and determination of

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body weight were made twice weekly. At the 29th day after initiation of the therapy, the animals were killed, the body weights were determined, and the uteri, ovaries, and adrenal glands were removed and weighed after having been treated as mentioned above.

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Supplementary Material Available: ^{1}H NMR data (Tables V and VI) and elemental analyses (Table VII) of compounds **7–32** (3 pages). Ordering information is given on any current masthead page.

Synthesis of Nitrosourea Derivatives of Pyridine and Piperidine as Potential Anticancer Agents

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Nitrosourea derivatives **18–22** which utilize either a piperidine or pyridine ring as a carrier group were synthesized and evaluated for anticancer activity. *N'*-(1-Benzyl-4-piperidinyl)-*N*-(2-chloroethyl)-*N*-nitrosourea hydrogen maleate (**19**) exhibited good activity against intracranial L1210 leukemia as well as the mouse ependymoblastoma brain tumor system. Compound **19** exhibited comparable activity in the Lewis lung carcinoma system to *N,N'*-bis(2-chloroethyl)-*N*-nitrosourea. Replacement of the *N*-benzyl group in both the 3-piperidinyl- and 4-piperidinylnitrosoureas resulted in less active compounds in all tumor systems tested. The 3-pyridylnitrosourea **22** was inactive in the L-1210 leukemia system.

The synthesis of a variety of nitrosoureas[2-5] have been described, many of which are highly active in mice against murine leukemia L1210 implanted either intraperitoneally[3-6] or intracerebrally.[4-6] Several nitrosoureas, including *N,N'*-bis(2-chloroethyl)-*N*-nitrosourea (BCNU, carmustine), *N*-(2-chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea (CCNU, lomustine), and *N*-(2-chloroethyl)-*N'*-(*trans*-4-methylcyclohexyl)-*N*-nitrosourea (MeCCNU, semustine), are currently undergoing extensive clinical trials.[7]

In a previous report, the synthesis and antileukemic activity of two ergoline nitrosoureas were described.[8] These compounds were designed in an attempt to combine antiprolactin and antitumor activity in the same molecule.

Based on the antitumor activity of these compounds, we have synthesized a series of related (based on the ergoline D ring) nitrosoureas which contain the pyridine and piperidine ring systems as carrier groups. These compounds are of considerable interest, since (a) they may be considered as nitrogen analogues of CCNU; (b) they form stable, water-soluble, crystalline salts; (c) the N-substituent may be altered in order to vary the lipophilicity of the compounds; and (d) they are less leukopenic than BCNU at equitoxic doses.

Chemistry. Piperidines containing the *N*-(2-chloroethyl)-*N*-nitrosourea group substituted at the 4 position of the ring (Table III) were prepared starting from ethyl isonipecotate. Precursors to the compound containing the nitrosourea group attached at the 4 position of the piperidine ring are given with their physical properties in Table I. The desired nitrosoureas **19** and **20** were prepared by nitrosation of the corresponding chloroethylureas **12** and **14** with sodium nitrite in 99% formic acid.[3] Isomeric purity was established by NMR spectroscopy (Table IV). The spectral symmetry[3] of the $ClCH_2CH_2N(NO)$ group (A_2B_2 system) is clearly shown in Table IV.

N-(1-Benzyl-3-piperidinyl)-*N*-(2-chloroethylurea) hydrogen maleate (**18**) was prepared starting from 3-acetamidopyridine (**7**). Catalytic reduction of **7** followed by alkylation with benzyl chloride and removal of the *N*-acetyl protecting group, gave the intermediate amine, **10**. The nitrosourea **18** was prepared by nitrosation of **11**.

A direct relationship between the lipophilicity of the N-substiuent and the yield of the nitrosourea seems apparent (Table III). A water-soluble hydrochloride of *N*-(2-chloroethyl)-*N'*-(1-methyl-4-piperidinyl)-*N*-nitrosourea has been reported.[9] However, our attempts to prepare nitrosoureas with a methyl group on the piperidine nitrogen gave unstable, highly water soluble products. The piperidinylchloroethylureas containing an *N*-ethyl or *N*-butyl group likewise gave complex reaction mixtures upon nitrosation.

Derivatives in which a (2-chloroethyl)urea group is substituted at the 2, 3, or 4 positions of pyridine were prepared in the normal manner, and their properties are given in Table II. Only *N*-(2-chloroethyl)-*N'*-(3-pyridyl)-*N*-nitrosourea (**22**) could be obtained by nitrosa-

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