Table V. Antidepressant Activity

Compd no.	Mouse approx LD_{50}, mg/kp ip	Dopa response mg/kg po	Dopa response Mouse act.[a]
2a	100	100	0
2a propionate	*b*	100	0
3a	300	100	+2
		25	0
3a propionate	*b*	100	0
2b	100	100	+2
		25	0
3b	300	100	+2
		25	0
2c	300	100	0
3c	300	100	0
2d	*b*	*b*	
3d	*b*	*b*	
2e	300	100	0
3e	300	100	0
2f	100	100	0
3f	300	100	+3
		25	+2
2g	300	100	+2
		25	0
3g	750	100	0
Imipramine		25	+3
		100	+3

[a]See ref 10. Ratings: 0 = inactive, +1 = slight potentiation, +2 = moderate, +3 = marked. [b]Not determined.

basic alumina. Elution was begun with petroleum ether at a flow rate of 5 ml/min. A combination of fractions gave 5.6 g (78%) of ester as an oil: ir (film) 3090, 3060, 3030 (arom), 2800, 2765, 2730 (Bohlmann), 1742 (ester carbonyl), 1176 (ester C-O), 769, 760, 702 cm^{-1} (arom); nmr (CCl_4) δ 1.00 (t, 3, CH_3), 2.20 (q, 2, CH_2), 7.20–7.48 (m, 2, arom), 7.52–7.90 (m, 2, arom).

3(e)-Phenyl-3(a)-propionoxyquinolizidine. The procedure described above was followed using 5.5 g (0.024 mol) of **2a**. The crude product was dissolved in 50 ml of 20% HCl and extracted with three 50-ml portions of Et_2O. The aqueous layer was cooled in an ice bath and basified to pH 11–13. The mixture was extracted with three 100-ml portions of Et_2O; the extract was dried over $MgSO_4$ and evaporated to give 4.9 g of a dark oil. The oil was chromatographed in a fashion similar to that described above to give 2.5 g (37%) of ester: ir (film, PE 137) 2750 (Bohlmann), 1740 (ester C=O), 1170 (ester C-O), 768, 756, 696 cm^{-1} (arom); nmr (CCl_4) δ 1.10 (t, 3, CH_3), 7.27 (s, 5, arom).

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Structure–Activity Relationships of the Mitomycins and Certain Synthetic Analogs

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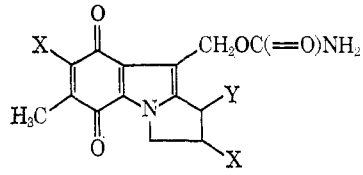
1-Acetoxy-7-methoxymitosene was highly active against gram-positive and gram-negative bacteria in cultures but inactive in mice. It is partly inactivated in the presence of serum, but simple binding to serum proteins does not account for this inactivation. 1-Hydroxy-7-methoxymitosene also was active against bacteria in culture but inactivated by serum. Both it and 2,7-diamino-1-hydroxymitosene were inactive against L1210 leukemia, despite the activity of the latter compound and related 1-hydroxymitosenes against certain sarcomas. Lipid-water partition coefficients were measured for these compounds, related mitosenes and indoloquinones, and naturally occurring mitomycins in order to establish structure-activity relationships. Only limited correlations could be made. These included antileukemia activities of the mitomycins, in which activity increased with hydrophilicity, and antibacterial activity of 7-methoxymitosenes and related indoloquinones, in which the best activity was obtained at moderate lipophilicity. The unique physicochemical properties of mitomycin C are cited as essential to future studies.

Recently we reported the synthesis of 1-substituted 7-methoxymitosenes including **1** and **2**.[1] These compounds are close structural analogs to mitomycin hydrolysis products such as apomitomycin A (**3**),[2] which has moderate antibacterial and antitumor activity.[3-5] As shown in Table I, compounds **1** and **2** are highly active against both gram-positive and gram-negative bacteria in culture. Thus **1** was one-fourth to one-eighth as active as mitomycin C (**9**) against many bacteria, whereas **2** was as potent as mitomycin C against *Diplococcus pneumoniae* and *Streptococcus pyogenes* but less active than **1** against gram-negative bacteria.

In contrast to its high activity against bacteria in cultures, **1** was inactive at doses of 20 mg/kg (administered intramuscularly at 1 and at 3.5 hr postchallenge) in mice infected with *S. pyogenes* A 9604 and with *Klebsiella pneumonia* A 9977. This disparity between the activity of **1** in culture and in mice was surprising, especially since the closely related 1-unsubstituted analog **6** is active against *Staphylococcus aureus* in mice but much less active than **1** in bacterial culture.[6] The observation (Table I) that both **1** and **2** have decreased activities against *S. aureus* in the presence of serum suggested that serum protein binding might be at least partly responsible for their inactivation. In order to investigate this possibility we measured the binding to bovine serum albumin (BSA) of **1**, **2**, **6**, and a variety of naturally occurring mitomycins and synthetic analogs which were included for comparison purposes. At the relatively high concentration of BSA (4%) used in this study, the bindings of **1** and **2** were 60

Table I. Antibacterial Activities of Mitosenes in Culture



Compd	X	Y	Z	Min inhib concn (μg/ml)[a] against: *D. pneu*	*Str. pyo*	*S. aur*	*S. aur* (+S)	*S. aur* (M)	*Sal. ent*	*E. coli*	*K. pneu*	*Pr. mor*	*P. aer*	*Ent. clo*
2	OCH_3	OH	H	0.008	0.008	0.06	0.25	0.25	16	125	16	16	na	125
1	OCH_3	OAc	H	0.03	0.03	0.03	0.13	0.13	4	8	2	0.5	8	8
5	NH_2	OH	H	2	2	8	8	16	na	na	125	na	na	na
7	NH_2	OH	NH_2	125	125	na	na	na	125	na	125	125	na	na
Mitomycin C (**9**)				0.008	0.008	0.03	0.03	0.13	0.25	1	0.25	0.13	2	4

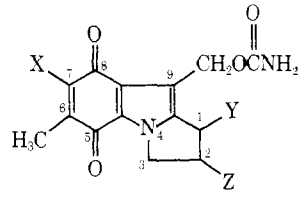
[a] Assays not all concurrent, but all standardized against mitomycin C. Compounds inactive at 125 μg/ml or lower indicated by na. Abbreviations for microorganisms: *D. pneu* = *Diplococcus pneumoniae* A 9585, *Str. pyo* = *Streptococcus pyogenes* A 9604, *S. aur* = *Staphylococcus aureus* strain Smith A 9537, (+S) = +50% serum, *S. aur* (M) = *Staphylococcus aureus* A 15097 (methicillin resistant), *Sal. ent* = *Salmonella enteritidis* A 9531, *E. coli* = *Escherichia coli* strain Juhl A 15119, *K. pneu* = *Klebsiella pneumoniae* A 9977, *Pr. mor* = *Proteus morganii* A 15153, *P. aer* = *Pseudomonas aeruginosa* A 9843A, *Ent. clo* = *Enterobacter cloacae* A 9656. For a complete description of the assay procedure, see M. Misiek, T. A. Pursiano, L. B. Crast, F. Leitner, and K. E. Price, *Antimicrob. Ag. Chemother.*, **1**, 54 (1972).

and 67%, respectively (Table II). Since these values are much lower than the 92% binding found for 6, it is concluded that serum protein binding is not the main cause for the decreased activity of 1 and 2 in the presence of serum.

One unanticipated but significant result of the protein binding study was the fact that mitomycin C (9) binds less strongly to BSA than any other compound in Table II. Since mitomycin C is a clinically important anticancer drug, we extended the study to include its binding to human serum proteins. The result of its binding as measured by an ultrafiltration method (Table III) was in good agreement with the result on binding to BSA. Binding of mitomycin C to human serum and plasma proteins by the agar diffusion method gave erratic results, due in part to enhancement of antibiotic activity by certain batches of plasma. However, all of these results indicated little or no binding of mitomycin C (Table III). We were unable to obtain any consistent results with an equilibrium dialysis method which was used previously to study the binding of mitomycin C and certain derivatives to BSA.[7]

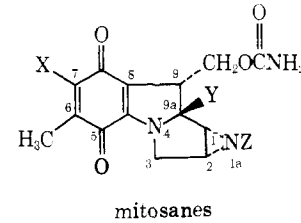
Since 1-hydroxymitosenes such as 3, 4, and 7 are reported to have moderate antitumor activities, we tested 1-hydroxy-7-methoxymitosene (2) against L1210 leukemia in BDF mice according to the CCNSC protocol. This test showed it to be toxic at 16 mg/kg/day but inactive at doses up to 6.4 mg/kg/day. In comparison, mitomycin C is active at 0.4 mg/kg/day in the same assay. A number of factors could be responsible for the inactivity of 2, including its lack of an aziridine ring and an unfavorable quinone reduction potential. Inactivation of 2 by serum might be another factor, although as shown in Table II it is not highly bound to BSA.

The recent report that a large majority of drugs effective against L1210 leukemia have log P values near to or less than zero[8] led us to study the lipid-water distribution properties of synthetic analogs such as 2 and compare them with those of the naturally occurring mitomycins. These partition coefficients are listed in Table II. It is apparent from this table that no simple broad generalizations concerning partition coefficients and biological activities can be made, but this could be due to the fact that a variety of structural types, in terms of quinone reduction potential and reactive functional groups, are present in this table. Within a structural type, certain useful correlations are possible. The most significant of these correlations is that among the natural mitomycins (all mitosane structures) the most hydrophilic compounds have the best activity against L1210 leukemia. Thus mitomycin C (log P = −0.38) and porfiromycin (**10**, log P = 0.03) are the most and next most active of the mitomycins against L1210 leukemia.[9] It should be noted that their log P values fit the "near to or less than zero" generalization for antileukemia activity.



mitosenes

1, X = CH_3O; Y = OAc; Z = H
2, X = CH_3O; Y = OH; Z = H
3, X = CH_3O; Y = OH; Z = NH_2
4, X = CH_3O; Y = OH; Z = NHAc
5, X = H_2N; Y = OH; Z = H
6, X = CH_3O; Y = Z = H
7, X = H_2N; Y = OH; Z = NH_2
8, X = H_2N; YZ = >NH

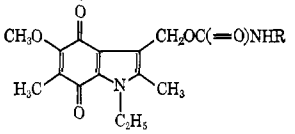


mitosanes

9, X = H_2N; Y = OCH_3; Z = H
10, X = H_2N; Y = OCH_3; Z = CH_3
11, X = CH_3O; Y = OCH_3; Z = H
12, X = CH_3O; Y = OH; Z = CH_3

Since, in addition to its other differences from mitomycin C, compound 2 is relatively lipophilic (log P =

Table II. Partition Coefficients and Serum Albumin Binding of Mitomycins

Compound	No.	Log P^a	% binding to protein[b]
Mitomycin A	**11**	0.26	75
Mitomycin B	**12**	0.11	
Mitomycin C	**9**	−0.38	29
Porfiromycin	**10**	0.03	52
Mitosenes			
7-OCH_3	**6**	1.71	92
7-OCH_3, 1-OH	**2**	1.10	67
7-OCH_3, 1-OAc	**1**	1.54	60
7-OCH_3, 1-OH, 2-NH_2	**3**	0.26	
7-NH_2, 1-OH	**5**	0.64	
7-NH_2, 1-OH, 2-NH_2	**7**	−0.20	56
Indoloquinones			
			
R = H	**13**	1.81	
R = C_2H_5	**15**	>2.6	
R = CH_2CH_2OH	**14**	1.64	
R = C_4H_9	**16**	>2.6	

[a]Distribution between 1-octanol and $^1/_{15}$ M phosphate buffer (pH 7.4) at 37°. Determined by a slight modification of the Hansch method (see Experimental Section). [b]Per cent of compound bound in 4% BSA solution at a free drug concentration of 1 mg % at 37°. Determined by an ultrafiltration procedure (see Experimental Section).

1.10), it became interesting to evaluate a structurally similar but more hydrophilic analog against L1210 leukemia. We chose 1-hydroxy-2,7-diaminomitosene (7) for this purpose because it is nearly as hydrophilic (log P = −0.20) as mitomycin C and it has been reported active against the Hirosaki ascites sarcoma.[10] It proved to be inactive at 50 mg/kg/day against L1210 leukemia, which shows that hydrophilicity alone is insufficient for activity. The need for an aziridine ring has already been stated as a prerequisite for antileukemia activity.[9]

Although the aziridine ring does not seem essential for antibacterial activity, as shown by the high activity of 1 in cultures and the oral activities of certain related mitosenes and indoloquinones,[6,11] the quinone reduction potential does strongly influence antibacterial activity. Thus the 7-methoxymitosenes (reduction potential −0.39 V) are more potent than the corresponding 7-aminomitosenes (−0.53 V), including even 7-aminoaziridinomitosene (8).[12] In the mitosane series, methoxyquinones (−0.19 V) also appear to be more active than aminoquinones (−0.395 V) although the differences are less significant.[3,8] Unfavorable reduction potentials probably account for the poor activities shown by 7-aminomitosenes 5 and 7 in bacterial cultures (Table I). Compound 7 had been reported previously to have antibacterial activity, but that result is questionable.[5]

Within the group of 7-methoxymitosenes and the 5-methoxyindoloquinones in Table II, a correlation of partition coefficients with antibacterial activities in cultures is possible since all of these compounds should have about the same quinone reduction potential. It appears that moderate lipophilicites produce the best activities since compounds 1 and 2 (log P = 1.54 and 1.10, respectively) are the most active, whereas the more lipophilic analog 6 (log P = 1.71) and the less lipophilic analog 3 (log P = 0.26) are both less active.[3,6] Indoloquinone 13 is a close analog of 6 and it has similar lipophilicity and antibacterial activity. The most active indoloquinone (14)[11] was the one with a lipophilicity (log P = 1.64) in the vicinity of that of compound 1.

Table III. Binding of Mitomycin C to Human Blood Proteins

% bound to blood proteins as determined by					
Agar diffusion method[a]			Ultrafiltration method[b]		
Specimen examined	Concn (μg/ml), antibiotic	% binding	Specimen examined	Concn (μg/ml), antibiotic	% binding
Plasma	0.5–3.0	0[c]			
Plasma	0.5–2.0	0			
Plasma	2–4	<10	Plasma	20	28
Serum	1–10	14–18	Plasma	20	26

[a]W. Scholtan and J. Schmid, *Arzniem.-Forsch.*, **12**, 741 (1962). [b]R. E. Buck, F. Leitner, and K. Price, *Antimicrob. Ag. Chemother.*, **1**, 67 (1972), with the following modifications: the CF-SOA filter cones were not used and the centrifugation process was omitted. Instead, all samples were filtered through the same Amicon ultrafiltration cell that was used for collecting deproteinized serum or plasma for the standard curve. [c]Activity enhanced in the presence of plasma.

In summary, we note that mitomycin C has a unique and unusual set of chemical and physical properties which are responsible for its biological activities. Thus it has good water solubility, low lipophilicity, and only minimal binding to serum proteins. Its quinone reduction potential is low enough to permit facile enzymic reduction, leading to activation of its two potent alkylating functions. The failure to date in developing an analog superior in clinical activity is due in part to the fact that most analogs have lacked one or more of the important physicochemical properties of mitomycin C. Proper attention to these unique properties should result in the design of more satisfactory analogs.

Experimental Section

Partition Coefficients. The lipid-water partition coefficients were determined by a procedure which was a slight variation from the standard Hansch method.[13] A 1-2-mg sample of each compound listed in Table II was weighed precisely on a Cahn electrobalance, dissolved in 0.5 ml of methanol, and diluted to 50 ml with M/15 phosphate buffer of pH 7.4 which had been previously saturated with 1-octanol. A portion of this solution was stirred for 90 min with a suitable proportion of 1-octanol previously saturated with the phosphate buffer, and then the layers were separated. Both the 1-octanol extracted and the nonextracted buffer solutions of the compound were centrifuged for 90 min. The necessity of this centrifugation was established by Hansch,[13] and it appears to involve the separation of minute droplets of octanol from the buffer solution. Ultraviolet absorption spectra were determined on a Cary Model 17 recording spectrophotometer for each of these buffer solutions and 1-octanol-water partition coefficients were calculated from the differences in optical density (OD) before and after partitioning with 1-octanol. They follow from the equation $P = (X_1 - X_2)/X_2(1/R)$, wherein X_1 = OD before partitioning and X_2 = OD after partitioning, and R is the ratio of the buffer volume to the 1-octanol volume. Due to their very low solubilities in the phosphate buffer, accurate partition coefficients could not be determined for compounds 15 and 16. They were estimated by comparing the OD of the compound in buffer after partitioning with the OD of the compound in 1-octanol, corrected for small differences in molar extinction coefficients in these two solvents.

Protein Binding Measurements. An ultrafiltration procedure was used for these measurements. Compounds were weighed, dissolved in 0.5 ml of methanol, and diluted to 50 ml with M/30 phosphate buffer at pH 7.4. A 20-ml portion of this solution was treated with 0.80 g of bovine serum albumin (a crystallized and

lyophilized preparation obtained from Sigma Chemical Co.) to make a 4% protein solution. This concentration was used by Hansch in a correlation of protein binding with partition coefficients.[14] Both the portions of solution with and without added protein were agitated at 37° for 16 hr. The protein-containing solution was filtered through an Amicon Model 12 ultrafiltration cell fitted with a Type UM 2 Diaflo ultrafiltration membrane. Both the filtrate and the solution without protein were measured by ultraviolet absorption spectroscopy. The per cent binding to BSA was determined by the equation: % binding = $(X_1 - X_2)/X_1(100)$, wherein X_1 = OD of solution not containing protein and X_2 = OD of filtrate after ultrafiltration. The OD values were measured at wavelengths above 310 nm since some absorption due to compounds from the BSA passing through the ultrafiltration membrane was found below 310 nm.

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Analgesics. 1. Selected 5-Substituted 5-Propionoxybarbituric Acids

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Several 5-substituted 5-propionoxybarbituric acids were synthesized and evaluated for analgesic activity. One compound, 5-propionoxy-5-(1-phenylethyl)barbituric acid (**29**), displayed better analgesic activity than codeine orally and had half the analgesic potency of morphine when administered subcutaneously. Compound **29** constitutes the *first example of a potent analgesic lacking a basic center* (of pK_a permitting extensive protonation at physiological pH).

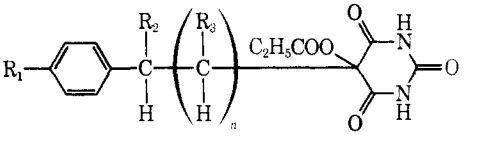
Several investigators have reported[1-3] the structural features generally found in potent analgesics; these may be summarized as follows: (1) a basic amine function, usually tertiary and limited in size; (2) a highly substituted central atom with none of its valency bonds linked to hydrogen (quarternary carbon or tertiary nitrogen); (3) an aromatic group (such as phenyl) connected to the central atom; (4) a two-carbon chain separating the central atom from the nitrogen.

During the past 15 years many potent analgesics have been described which possess structural features that deviate from the above requirements, particularly with respect to the permitted size of the basic group, and the necessity for a reevaluation of structure-activity relationships in analgesics was pointed out as early as 1959 by Eddy.[4]

However, as late as 1970 Casy[5] stated that "in spite of the continual appearance of novel structures characterized as having morphine-like actions which are reversed by antagonists such as nalorphine, no significant analgesic has been yet identified which lacks either a *basic center* (of pK_a permitting extensive protonation at physiological pH values) or *aromatic* features."

We have now synthesized a series of compounds which are characterized by the following structural features: (1) the lack of a basic center (of pK_a permitting extensive protonation at physiological pH values); (2) the lack of a central quaternary carbon atom to which a phenyl group is connected; (3) the lack of a two-carbon chain separating a central quaternary carbon atom from the nitrogen atom.

The general structure of the new class of potent analgesics is shown



where R_1 = H, NO_2, Cl, MeO; R_2 = H, Me, Et; n = 0, 1. When n = 1, R_3 = H, Me.

†A preliminary account of this work was presented by J. A. Vida and C. M. Samour before the Medicinal Chemistry Division, 165th National Meeting of the American Chemical Society, Dallas, Texas, April 1973, Abstracts of Papers, MEDI-21.