6.62 (2 s, 1 each, aromatic); mass spectrum *m/e* (rel intensity) M^+ 515 (22), 484 (4), 314 (20), 298 (100), 282 (12), 266 (10), 228 (20), 173 (24), 150 (12), and 99 (40). Found: M^+, 515.245; $C_{28}H_{37}NO_8$ requires 515.252.

Compound **1b** was treated with the silver salt of **6** as described: yield of **2b** by preparative tlc, 56%. Its ir spectrum was identical with that of **3**: uv λ_{max} (EtOH) 292 nm (ϵ 4895); $[\alpha]^{26}D$ −155.7° (*c* 0.42, $CHCl_3$); nmr δ 0.86 (d, 6, J = 6 Hz, isopropyl), 3.55 (s with shoulder,†† 3, allylic methoxyl), 3.62 and 3.65 (2 s,†† 3, carbomethoxyl), 3.95 (2 dd,†† 1, $J_{1,2}$ = 2 Hz, $J_{2,3}$ = 2 Hz, on C-2), 4.63 (d, 1, J = 2 Hz, vinyl), 5.85 (s, 2, methylenedioxy), 6.00 and 6.04 (2 d,†† 1, J = 2 Hz, on C-1), and 6.58 (br s, 2, aromatic); mass spectrum *m/e* (rel intensity) M^+ 515 (40), 484 (10), 314 (36), 298 (100), 282 (9), 266 (7), 228 (38), 173 (16), 150 (8), and 99 (24). Found: M^+, 515.251; $C_{28}H_{37}NO_8$ requires 515.252.

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Preparation and Antileukemic Activity of Some Alkoxybenzo[*c*]phenanthridinium Salts and Corresponding Dihydro Derivatives[1]

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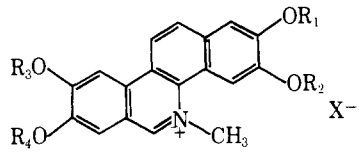
Salts of 2,3,8,9-tetrasubstituted alkoxy-, hydroxy-, and acetoxybenzo[c]phenanthridines as well as the corresponding 6-methoxy-5,6-dihydrobenzo[c]phenanthridines were prepared from appropriate chalcones through the tetralone and the 4b,10b,11,12-tetrahydrobenzo[c]phenanthridine intermediates. Complete O-demethylation of the tetramethoxybenzophenanthridine was achieved by fusion with pyridine hydrochloride at elevated temperature. The title compounds are active against leukemias L1210 and P388 in mice and some are curative against Lewis lung carcinoma. The importance of the nature of the environment about the nitrogen atom of these compounds and the substituents is discussed. 3,4-Dimethoxy-3′,4′-methylenedioxychalcone possesses activity against leukemia P388.

A number of alkoxybenzophenanthridine alkaloids have demonstrated interesting biological and pharmacological activities. Among these, nitidine chloride[2-4] (**1a**, X = Cl) and 6-methoxy-5,6-dihydronitidine[3,4] (**2a**), isolated from *Fagara macrophylla*, were shown to be highly cytotoxic, displayed antileukemic activity in both leukemia L1210 and P388 systems in mice, and inhibited Lewis lung carcinoma. Fagaronine[5] (**1b**), isolated from *Fagara zanthoxyloides* Lam., showed good activity against leukemia P388. This paper presents the preparation and antileukemic activity of several 2,3,8,9-tetrasubstituted benzophenanthridinium salts related to nitidine and allied compounds.[4,6]

Chemistry. The general synthetic route leading to the substituted benzo[*c*]phenanthridine ring system **5** follows essentially the same procedure as that used for the synthesis of nitidine[4] and allonitidine.[6] The overall yields of the nine-step synthesis of benzo[*c*]phenanthridinium salts **1** from the appropriate acetophenones through chalcones **3** and the tetrahydro intermediates **4** were 9–15%. Treatment of **1** in cold NH_4OH followed by heating the intermediate with CH_3OH afforded the methoxyl derivatives of dihydrobenzophenanthridine (**2**).

It has been postulated that the alkoxyl functions on compounds **1** and **2** may have to be dealkylated *in vivo* for necessary biological action. Consequently, preparation of the tetrahydroxy (**1e**) and the tetraacetoxy (**1f**) analogs of nitidine was conducted. Demethylation of 2,3,8,9-tetramethoxybenzo[*c*]phenanthridine (**5d**) was studied with a variety of known demethylating agents including 30% HBr in AcOH,[7,8] BBr_3 in $CHCl_3$,[9,10] and pyridine hydrochloride.[11,12] None of these agents under ordinary reaction conditions yielded completely demethylated product. For example, refluxing a mixture of **5d** and pyridine hydrochloride in nitrobenzene for extended periods only resulted in cleavage of two methoxy groups. However, when the aforementioned reactants were fused at 280° for 3 hr, complete demethylation took place with simultaneous N-methylation, and the yield of compound **1e** (X = Cl), complexed with 2 equiv of pyridine hydrochloride, was practically quantitative.

Acetylation of **1e** was accomplished in over 80% yield by heating the compound with a mixture of Ac_2O and pyridine for 2 hr at 145–150°. The product, **5f**, could not be methylated under the usual reaction conditions used for



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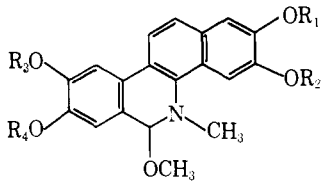

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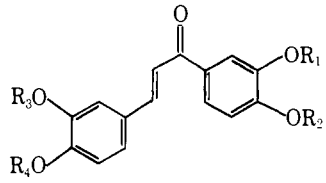

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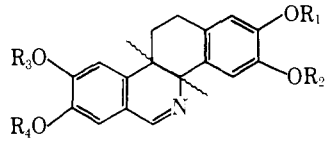

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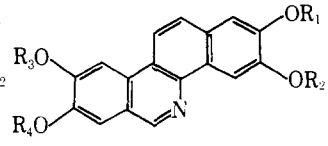


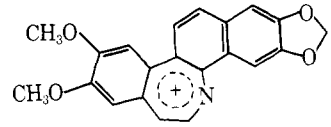
5

a, $R_1 + R_2 = CH_2$; $R_3 = R_4 = CH_3$
b, $R_1 = H$; $R_2 = R_3 = R_4 = CH_3$
c, $R_1 = R_2 = CH_3$; $R_3 + R_4 = CH_2$
d, $R_1 = R_2 = R_3 = R_4 = CH_3$
e, $R_1 = R_2 = R_3 = R_4 = H$
f, $R_1 = R_2 = R_3 = R_4 = COCH_3$

the preparation of nitidine or allonitidine. Apparently, the nitrogen atom in **5f** under the influence of four acetoxyl groups was not basic enough to be methylated by methyl sulfate. Since fluorosulfonic acid is known to be one of the strongest acids, alkylation with methyl fluorosulfonate may offer a solution to this difficulty. Thus, the commercially available Magic Methyl (Aldrich) was used. The desired methylation was successfully carried out under quite strict reaction conditions, *i.e.*, at 110–120° for 10 min using nitrobenzene or a mixture of nitrobenzene and xylene. The strictness of reaction conditions can be demonstrated by the fact that no quaternary salt formation was observed at reaction temperature below 110° and, at temperatures higher than 140° or longer reaction times, cleavage of all acetyl groups occurred. The desired compound **1f** (X = SO_3F) was obtained as a greenish yellow solid.

A comparison of the structure of nitidine (**1a**) with that of another antileukemic alkaloid coralyne[13,14] (**6**) logically suggested the synthesis of compound **7a** wherein a methyl group is substituted at the 6 position of the benzo[*c*]-phenanthridine series. This compound was prepared by heating the acetylated oxime **8b** in a sealed bottle with a mixture of Ac_2O and AcOH presaturated with dry HCl.[15] This procedure serves as a convenient synthesis of 6-alkylated benzo[*c*]phenanthridines.

Detailed mechanism for the formation of compound **7a** is not yet known. It appears that the following steps were involved in a single treatment: (a) reduction of the acetoxime to an amine;[15-17] (b) acetylation of the amine; (c) aromatization of the tetrahydronaphthalene ring; and (d) cyclization of the acetamidonaphthalene intermediate to the benzo[*c*]phenanthridine ring system. The postulation was supported by the isolation of the aromatized acetamido compound[9] from the reaction mixture. The importance of participation of the acid anhydride during this reaction was substantiated by the fact that the corresponding ethyl homolog **7b** can be prepared from a mixture of the acetoxime **8b**, AcOH, propionic anhydride, and gaseous HCl. It is of interest to note that mass spectrum determination of **7b** gave *m/e* 361 (90%, $M^+ - HCl$), 346 (100%, $M^+ - CH_3 - HCl$). The high percentage of *m/e* 346 (the intensity is higher than the parent molecular ion 361) suggests formation of a relatively stable azotropylium ion 10 during the process of electronic fragmentation. Similar formation of azotropylium fragments from quinine or other quinoline and isoquinoline alkaloids during mass spectrum determination has also been noted.[18]



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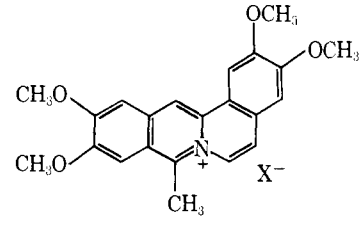

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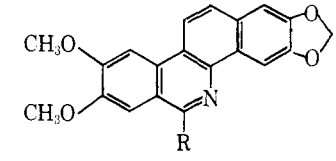
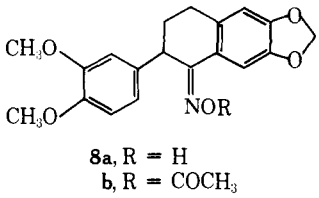
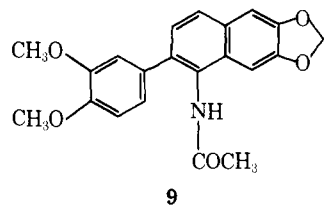


7a, R = CH_3
b, R = C_2H_5



8a, R = H
b, R = $COCH_3$



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As in the case of coralyne[14,19] (**6**), the uv absorption peak of nitidine chloride (**1a**, X = Cl) in aqueous solution shifted rapidly from 272 to 262 nm on standing. This is probably due to multiple bond hydration across the C=N bond to form a 5,6-dihydro compound. The hydration seems to be reversible since, when the aqueous solution of nitidine chloride was kept at room temperature for 2 hr and then lyophilized, the resulting solid showed the same melting point and uv spectrum as the original compound. It was noted that anhydrous CH_3OH did not add across the C=N bond under similar conditions.

For the purpose of improving aqueous solubility and stability in formulation, conversion of nitidine chloride into other salts has also been studied. This was found to be readily achieved through the methoxydihydro intermediate **2a**.

Biological Results and Discussion. Preliminary test results of these compounds indicated that (a) salts of nitidine (**1a**), allonitidine (**1c**), and the tetramethoxy analog **1d** possessed antileukemic activity against both leukemias L1210 and P388; (b) nitidine chloride (**1a**, X = Cl) and 6-methoxy-5,6-dihydronitidine (**2a**) showed curative activity against Lewis lung carcinoma; (c) antileukemic activity of the tetramethoxy analog **1d** seems to be slightly greater than that of nitidine (**1a**), which, in turn, was slightly better than that of allonitidine **1c**; as in the case

Table I. Antileukemic Activity of Alkoxybenzo[*c*]phenanthridines and Related Compounds

Compound	Mol formula	Antileukemic activity[a] P388 Dose, mg/kg	Survival	Wt diff	*T/C*, %	L1210 Dose, mg/kg	Survival	Wt diff	*T/C*, %
1a (X = Cl)[b]	$C_{21}H_{18}ClNO_4$	100	6/6	−0.8	129	100	3/3	−0.8	111
		80	18/18	−2.2	194	64	6/6	−3.6	121
		32	36/36	−2.1	189	32	6/6	−1.3	127
		16	36/36	−1.5	174	16	6/6	−1.3	119
		8	16/16	−1.1	190	8	6/6	−1.1	127
		4	28/28	−0.7	172	4	6/6	−0.6	124
		2	42/42	−0.4	146	2	6/6	−0.5	119
		1	36/36	−0.4	123				
1a (X = Cl) (natural product)	$C_{21}H_{18}ClNO_4$	8	10/10	−1.5	209	8	20/20	−1.7	130
		4	10/10	−0.9	209	4	20/20	−0.7	136
		2	20/20	−0.3	177	2	20/20	−0.4	131
		1	20/20	−0.3	155	1	20/20	−1.1	127
1a (X = CH_3SO_4)	$C_{22}H_{21}NO_6S$	8	10/10	−0.5	176				
		4	10/10	−0.6	161				
		2	10/10	−0.2	142				
		1	10/10	−0.5	133				
1c (X = CH_3SO_4)	$C_{22}H_{21}NO_6S$	240	7/12	−5.5	55	80	6/6	−2.9	142
		120	12/12	−3.2	106	40	6/6	−1.9	122
		80	12/12	−3.2	113	20	6/6	−1.4	112
		40	12/12	−2.7	157	10	6/6	−1.3	124
		20	12/12	−1.4	131				
1d (X = Cl)	$C_{22}H_{22}ClNO_4$	400	16/18	−2.6	143	200	6/6	−2.6	151
		240	12/12	−1.6	147	100	6/6	−0.9	134
		200	12/12	−2.5	139	50	6/6	0.4	131
		100	18/18	−1.9	155				
		50	12/12	−1.7	165				
		25	6/6	0.1	142				
		12.5	6/6	−1.2	134				
1d (X = CH_3SO_4)	$C_{23}H_{25}NO_6S$	240	6/6	−0.3	90	270	6/6	−4.4	144
		120	12/12	−0.8	162	180	12/12	−3.9	140
		80	12/12	−3.0	168	120	12/12	−2.4	140
		52	12/12	−0.9	150	80	12/12	−2.0	141
		40	12/12	0.0	145	52	12/12	−1.1	137
		20	6/6	−1.0	140	34	12/12	−0.9	133
1d (X = SO_3F)	$C_{22}H_{22}FNO_7S$	400	6/6	−0.9	92	666	3/6	−3.1	63
		200	6/6	−0.4	174	400	6/6	−1.6	121
		100	6/6	−0.6	148	200	6/6	−1.4	113
1e (X = Cl)	$C_{18}H_{14}ClNO_4 \cdot 2C_5H_5N \cdot 2HCl$					240	6/6	−2.5	98
						120	6/6	−1.1	98
						80	6/6	−0.8	107
						40	6/6	−0.6	102
1f (X = SO_3F)	$C_{23}H_{22}FNO_{11}S$	240	5/6	−4.7	73	120	6/6	−2.0	91
		120	6/6	−1.8	118	80	6/6	−1.2	104
		80	6/6	−1.1	109	40	6/6	0.4	109
		40	6/6	−0.7	104	20	6/6	−0.7	101
2a[c]	$C_{22}H_{21}NO_5$	120	6/6	−1.7	175	180	6/6	−4.0	156
						120	6/6	−4.5	175
		52	6/6	−1.6	181	80	6/6	−8.3	147
		40	6/6	−1.1	175	50	6/6	−1.8	147
		34	6/6	−1.3	145	34	6/6	−1.1	147
		20	6/6	−0.3	165				
2a (natural product)	$C_{22}H_{21}NO_5$	80	12/12	−2.5	180	80	11/12	−1.3	136
		40	16/16	−3.5	194	40	25/26	−2.1	133
		20	16/16	−3.5	207	20	26/26	−1.7	138
		10	15/16	−2.6	188	10	20/20	−1.2	126
		5	16/16	−1.4	186	5	14/14	−0.9	131
2c	$C_{22}H_{21}NO_5$	240	12/12	−2.5	95	240	6/6	−4.2	111
		120	12/12	−0.4	138	120	6/6	−3.7	119
		80	12/12	−0.4	132	80	6/6	−1.7	128
		40	12/12	−1.2	145	40	6/6	−0.2	124
		20	12/12	0.0	133	20	6/6	−0.7	119
						10	6/6	−0.4	125

Table I *(Continued)*

Compound	Mol formula	Antileukemic activity[a] P388 Dose, mg/kg	Survival	Wt diff	T/C, %	L1210 Dose, mg/kg	Survival	Wt diff	T/C, %
2d	$C_{23}H_{25}NO_5$	400	5/6	−2.5	102	400	18/18	−1.8	113
		300	6/6	−0.6	166	200	24/24	−1.1	129
		200	11/12	−0.6	155	100	24/24	−1.1	123
		100	11/12	−0.5	154				
		50	6/6	0.0	134				
3a	$C_{18}H_{16}O_5$	400	6/6	−4.7	194	400	12/12	−0.2	95
		300	12/12	−1.6	143	200	12/12	0.3	94
		200	18/18	−2.2	143	100	12/12	−0.1	96
		150	6/6	−0.1	127				
		100	12/12	−0.8	140				
		88	12/12	−0.7	125				
		66	6/6	−0.9	118				
3c	$C_{18}H_{16}O_5$					400	12/12	−0.5	93
						200	12/12	0.0	94
						100	11/12	0.1	93
3d	$C_{19}H_{20}O_5$	400	12/12	−1.0	107	400	12/12	−0.5	100
		200	12/12	−1.0	100	200	12/12	−0.4	103
		100	12/12	0.0	90	100	12/12	0.4	98
4a	$C_{20}H_{19}NO_4$	360	6/6	−1.6	105	400	6/6	−0.8	91
		120	12/12	0.2	102	240	6/6	−1.1	98
		40	6/6	−1.1	105	80	6/6	−0.4	96
4d	$C_{21}H_{23}NO_4$	360	6/6	−1.8	105	360	6/6	−1.8	93
		240	12/12	−0.9	100	240	6/6	−1.0	93
		80	6/6	−1.5	100	80	6/6	−0.6	102
5a	$C_{20}H_{15}NO_4$	360	6/6	−4.5	100	400	6/6	−3.8	90
		120	12/12	−2.0	102	240	6/6	−3.5	102
		40	6/6	0.1	105	80	6/6	−3.1	101
5c	$C_{20}H_{15}NO_4$	400	6/6	−1.2	92	400	12/12	−1.5	98
		100	6/6	0.3	83	200	12/12	−1.0	92
		25	6/6	0.5	95	100	12/12	−0.5	95
5d	$C_{21}H_{19}NO_4$	360	6/6	−1.8	120	400	5/6	−1.3	83
		240	12/12	−1.0	105	240	6/6	−1.7	91
		120	12/12	−1.8	105	80	6/6	−0.2	88
		80	12/12	−0.4	108				
5f	$C_{25}H_{19}NO_8$	240	6/6	−2.4	95				
		80	12/12	−1.3	84				
		20	12/12	−0.9	82				

[a] For general screening procedure and data interpretation, *cf.* R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep.*, 3 (2), 1 (1972); Instruction Booklet 14, "Screening Data Summary Interpretation and Outline of Current Screen," Drug Research and Development, Chemotherapy, National Cancer Institute, Bethesda, Md., 1973. [b] Against Lewis lung carcinoma: 3/10 cured at 50 mg/kg; +KB (dose required for ED_{50}: 7.5×10^{-2} μg/ml). [c] Against Lewis lung carcinoma: 3/10 cured at 200 mg/kg and 1/10 cured at 50 mg/kg; +KB (dose required for ED_{50}: 1.0×10^{-2} μg/ml).

of the coralyne series,[14] activity is often manifested at lower dose levels; (d) the tetrahydroxy (**1e**) and the tetraacetoxy (**1f**) analogs of nitidine were inactive; (e) none of the uncharged parent ring systems (**5**) which were very insoluble in water, or the corresponding 4b,10b,11,12-tetrahydro intermediates were active; (f) the antileukemic activity of 6-methoxy-5,6-dihydro analogs (**2**) was slightly higher than the corresponding salts (**1**); (g) one of the alkoxychalcones, 3,4-dimethoxy-3′,4′-methylenedioxychalcone (**3a**), possessed activity against leukemia P388 (see Table I).

The synthetic nitidine chloride (**1a**, X = Cl) and 6-methoxy-5,6-dihydronitidine (**2a**) possessed the same level of antileukemic activity as the corresponding naturally isolated counterparts;[3] thus the antileukemic property cannot be due to the presence of any possible minor constituents in the natural products. Although the active species or the mode of action of **1** and **2** are not yet understood, it appears that the environmental effect around the nitrogen atom of these compounds plays an important role in biological activity. This could either be due to some *in vivo* hydration characteristics of these molecules, alkylation or addition at the 6 position by certain biological species, or formation of a reactive center at or near the nitrogen atom. Both compounds **1** and **2** are unstable on standing in water or dilute acid, and the parent ring **5** gradually precipitates from the aqueous solution. This property may be significant for the *in vivo* slow release of certain active species at the binding site. For the purpose of biological screening, it is therefore very important that freshly prepared solutions of **1** or **2** be tested.

The fact that the uncharged parent rings **5** and the tetrahydroxy compound **1e** are inactive in the screening tests does not necessarily mean that these compounds may not be the active species or intermediates *in vivo*. It may rather be due to their extreme insolubility in water, which

may hinder the *in vivo* transport of these materials to the desired sites. It is of interest to note that compounds of types 1 and 2, including 1e and 1f, which did not show activity *in vivo* for the reasons already discussed, were found to be good inhibitors of catechol *O*-methyltransferase[20,21] and tRNA methylases[22] in *in vitro* tests (unpublished work). A possible correlation between inhibition of tRNA *O*-methyltransferase and antineoplastic activity has earlier been postulated in this laboartory.[23]

Certain hydroxy- and dimethylaminomethyl-substituted chalcones were reported to possess inhibitory action against Ehrlich ascites tumor or Sarcoma-180 in mice.[24,25] Among the series of chalcones prepared as intermediates for the present study, only 3,4-dimethoxy-3′,4′-methylenedioxychalcone demonstrated activity against leukemia P388. The structure of this compound bears some resemblance to that of many antineoplastic compounds such as cytembena,[26-29] β-(2-nitrovinyl)naphthalene and related nitrovinyl derivatives,[30] and many α,β-unsaturated lactones.[31,32] Presumably an activated double bond adjacent to an electron-withdrawing group may facilitate certain addition or alkylation reactions *in vivo* which, in turn, are responsible for the intriguing biological activity.

Experimental Section

All melting points were taken on a Thomas-Hoover melting point apparatus. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

Preparation of compounds 1a,c,**d** and 2a,c,**d** has been reported.[4-6]

8,9-Dimethoxy-6-methyl-2,3-methylenedioxybenzo[*c*]phenanthridine (7a). A mixture of 3 g of 1-acetoxyimino-2-(3,4-dimethoxyphenyl)-6,7-methylenedioxy-1,2,3,4-tetrahydronaphthalene (**8b**) in 15 ml of AcOH and 22 ml of Ac_2O (presaturated with dry HCl at 0°) was heated in a pressure bottle on a steam bath for 8 hr. The reaction mixture was cooled and diluted with Et_2O, and the resulting yellow solid (2.8 g, mp *ca.* 190°) was collected by filtration. It consisted of a mixture of **7a**·HCl and the acetamide intermediate **9**, along with some minor impurities. The solid was treated with methanolic NH_3 and recrystallized from a mixture of MeOH–C_5H_5N to give 0.66 g (28% yield) of **7a**: mp 230–232° (lit.[15] mp 233°); λ_{max} ($CHCl_3$) 280 nm (log ε 5.11), 305 (sh, 4.64), 330 (4.29), 349 (3.99), and 366 (3.74); R_f 0.50 (SiO_2, $CHCl_3$), 0.70 (Al_2O_3, $CHCl_3$). *Anal.* ($C_{21}H_{17}NO_4$) C, H, N.

The HCl salt of **7a** melted at 297–298°. *Anal.* ($C_{21}H_{17}NO_4$·HCl·0.5H_2O) C, H, N.

Attempted quaternization of **7a** using MeI or Me_2SO_4, under standard conditions or in a sealed bottle, was not successful.

From the mother liquor of **7a**, 0.6 g (26% yeild) of the acetamido intermediate **9** was isolated: mp 210–211°; λ_{max} (EtOH) 235 nm (log ε 4.74), 251 (4.74), 285 (4.24), 322 (3.86), and 335 (3.92); *m/e* 365 (M^+, 100%), 323 ($M^+ - COCH_3$, 100%); R_f 0.30 (SiO_2, $CHCl_3$), 0.55 (Al_2O_3, $CHCl_3$). *Anal.* ($C_{21}H_{19}NO_5$) C, H, N.

8,9-Dimethoxy-6-ethyl-2,3-methylenedioxybenzo[*c*]phenanthridine (7b) was prepared in a fashion similar to that used for **7a** except that propionic anhydride rather than Ac_2O was used. The HCl salt of **7b** was isolated in 13% yield: mp 280–281°; *m/e* 361 ($M^+ - HCl$, 90%), 346 ($M^+ - HCl - CH_3$, 100%). *Anal.* ($C_{22}H_{19}NO_4$·HCl) C, H, N.

2,3,8,9-Tetraacetoxybenzo[*c*]phenanthridine (5f). A mixture of 0.5 g of **5d** (0.14 mmol) and 10 g of pyridine hydrochloride was heated under N_2 at 280–285° for 3 hr. The reaction mixture (containing 1e) was cooled and 20 ml of pyridine added. To this was then added 40 ml of Ac_2O and the mixture again heated at 145–150° for 2 hr with stirring. It was cooled and allowed to stand overnight. The resulting crystalline product was collected by filtration, washed with EtOH (2 × 10 ml) and H_2O (2 × 10 ml), and dried to give 0.53 g (80% yield) of the desired tetraacetoxy compound **5f**, mp 288–291°. An analytical sample (needles) was prepared by recrystallization from EtOH: mp 291–293°. Its mass spectrum showd no residual methoxyl fragments: *m/e* 461 (M^+); λ_{max} (EtOH) 242 nm (log ε 4.41), 260 (4.77), 268 (4.96), 342 (3.66), and 361 (3.57). *Anal.* ($C_{25}H_{19}NO_8$) C, H, N.

When the initial demethylation reaction with pyridine hydrochloride was carried out in $C_6H_5NO_2$ at 210°, only two methoxyl groups were cleaved.

5-Methyl-2,3,8,9-tetraacetoxybenzo[*c*]phenanthridinium Fluorosulfonate (1f, X = SO_3F). To a warm solution of 0.46 g (0.1 mmol) of **5f** in 15 ml of $C_6H_5NO_2$ at 110–120° was added 1 ml of methyl fluorosulfonate (Magic Methyl, Aldrich). The mixture was stirred at that temperature for 10 min whereupon a yellow solid precipitated. The reaction mixture was cooled and diluted with 100 ml of Et_2O. The solid was collected by filtration, washed with ether (3 × 30 ml), and dried to give 0.60 g (quantitative yield) of the desired product, mp 233–235° dec. The product was slightly soluble in H_2O and soluble in MeOH. The aqueous solution was unstable. It gave a delayed positive $FeCl_3$ test on standing: λ_{max} (MeOH) 222 nm (log ε 4.51), 269 (4.69), 315 (4.21), and 412 (3.81). *Anal.* ($C_{26}H_{22}FNO_{11}S$) C, H, N.

5-Methyl-2,3,8,9-tetramethoxybenzo[*c*]phenanthridinium Fluorosulfonate (1d, X = SO_3F). To a hot (150–160°) solution of 0.8 g (0.22 mmol) of **5d** in a mixture of 30 ml of $C_6H_5NO_2$ and 15 ml of xylene was added 2 ml of methyl fluorosulfonate. Almost immediately a yellow product separated. The mixture was stirred at room temperature for 45 min and cooled. The solid was collected by filtration, washed with ether (3 × 50 ml) and petroleum ether (3 × 50 ml), and dried to give 1.1 g (quantitative yield) of **1d** (X = SO_3F) as a hemihydrate: mp 333–335° dec. *Anal.* ($C_{22}H_{22}FNO_7S$·0.5H_2O) C, H, N.

5-Methyl-2,3,8,9-tetrahydroxybenzo[*c*]phenanthridinium Chloride (1e, X = Cl). A mixture of 0.6 g (0.17 mmol) of 2,3,8,9-tetramethoxybenzo[*c*]phenanthridine[6] (**5d**) and 10 g of pyridine hydrochloride was heated at 280–285° under N_2 for 3 hr. The cooled reaction mixture was stirred with 80 ml of MeOH for 5 min. The product was collected by filtration and washed with MeOH (2 × 20 ml) and ether (2 × 20 ml) to give 0.5 g of a yellow solid, mp >360°. The product, which was isolated as a complex with 2 equiv of pyridine hydrochloride and was soluble in H_2O, was purified by recrystallizing from EtOH containing 10% pyridine: mp >360°; λ_{max} (MeOH) 236 nm (log ε 4.62), 271 (4.92), 330 (4.54), and 385 (4.17). *Anal.* ($C_{18}H_{14}ClN$·2C_5H_5N·2HCl) C, H, N.

Conversion of Nitidine Chloride (1a, X = Cl) to Nitidine Methyl Sulfate (1a, X = CH_3SO_4). To 120 ml of 28% NH_4OH cooled at 0–5° was added, with stirring, 1 g of nitidine chloride dihydrate. The mixture was stirred in an ice bath for 15 min and then extracted with $CHCl_3$ (4 × 200 ml). The $CHCl_3$ extract was dried (Na_2SO_4) and evaporated under reduced pressure at <40°. The residue was dissolved in 200 ml of warm (45–55°) MeOH and a trace of insoluble substance was removed by filtration. To the cold filtrate, which contained the intermediate 6-methoxy-5,6-dihydronitidine (**2a**), was added dropwise 10% (v/v) of Me_2SO_4 in MeOH (*ca.* 45 ml was added to pH 2). The resulting yellow precipitate was collected by filtration and washed with MeOH (20 ml) and petroleum ether (2 × 80 ml). It was dried at room temperature to give 0.88 g (77% yield) of the methyl sulfate salt, mp 311–312°. The ir and uv of the product were found to be identical with those of nitidine methyl sulfate prepared by an earlier method.[6]

Under similar conditions, the following salts were obtained through the dihydronitidine intermediate **2a**. **Nitidine chloroacetate (1a, X = $ClCH_2CO_2$)**: 90% yield; mp 274–276°. *Anal.* ($C_{23}H_{20}ClNO_6$·0.5H_2O) C, H, N. **Nitidine nitrate (1a, X = NO_3)**: 90% yield; mp 278–280°. *Anal.* ($C_{21}H_{18}N_2O_7$) C, H, N. **Nitidine acetosulfate (1a, X = CH_3COSO_4)**: 87% yield; mp 268–270°. *Anal.* ($C_{23}H_{21}NO_9S$·H_2O) C, H, N.

Among these nitidine salts, the chloroacetate was found to be more soluble in water than other salts prepared: solubility for the chloroacetate salt in water, 1 mg/ml; for the chloride salt, 0.2 mg/ml at room temperature.

Conversion of 5-Methyl-2,3,8,9-tetramethoxybenzo[*c*]phenanthridinium Methyl Sulfate (1d, X = CH_3SO_4) to 5-Methyl-2,3,8,9-tetramethoxybenzo[*c*]phenanthridinium Chloride (1d, X = Cl). A slurry of 8 g (1.69 mmol) of powdered **1d** (X = CH_3SO_4)[6] in 1500 ml of water was added to 1500 ml of 10% aqueous NaCl solution at 0–5°. The mixture was stirred at that temperature for 30 min and the solid was collected by filtration. It was washed with H_2O (40 ml) and ether (3 × 50 ml) and dried, then recrystallized from warm MeOH to give **1d** (X = Cl) as yellow crystals: mp 303–305° (lit.[33] mp 293–295°). The yield was quantitative.

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Quinuclidine Chemistry. 3.[1] β-*cis*-2-(4′-Chlorobenzhydryl)-3-quinuclidinol, a New Central Nervous System Stimulant. Importance of the Benzhydryl Configuration

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The 1,4 addition of *p*-chlorophenylmagnesium bromide to 2-benzylidene-3-quinuclidinone gave 2-(4-chlorobenzhydryl)-3-quinuclidinone as two diastereoisomers. Selective reduction of this ketone with aluminum isopropoxide gave the two *cis*-2-(4-chlorobenzhydryl)-3-quinuclidinols, which differ only in the configuration of the benzhydryl group, designated α and β in order of their elution on chromatography. Reduction with $NaBH_4$ gave a mixture of four isomeric alcohols, of which the two cis isomers were selectively oxidized. The two *trans*-2-(4-chlorobenzhydryl)-3-quinuclidinols were chromatographically separated and designated α and β in order of elution. Only the β-cis and β-trans alcohols showed CNS stimulant properties. The β-cis isomer was shown to be related both qualitatively and quantitatively more to methylphenidate (Ritalin) than to *d*-amphetamine.

Our interest in quinuclidines as medicinal agents led to the discovery of the antiinflammatory properties of *cis*-2-(4,4′-difluorobenzhydryl)-3-quinuclidinol.[1] This compound was devoid of any effects on the central nervous system (CNS). We now report the synthesis of a potent CNS stimulant, β-*cis*-2-(4′-chlorobenzhydryl)-3-quinuclidinol (**2**), in which activity is critically dependent upon the configuration of the benzhydryl moiety.

Chemistry. The reaction of 2-benzylidene-3-quinuclidinone[2,3] with *p*-chlorophenylmagnesium bromide (see Scheme I) gave, by 1,4 addition, the ketone **1** as a mixture of two diastereoisomers. Reduction of **1** with aluminum isopropoxide under conditions where the resulting acetone was immediately removed to prevent equilibration gave selectively the two isomeric cis alcohols **2** in approximately equal amounts as detected by tlc analysis. The cis configuration is a consequence of hydride transfer to the carbonyl from the least hindered side, *i.e.*, trans to the benzhydryl group.† These isomers were separated by column chromatography and were designated 2α and 2β in order of their elution. These alcohols differ only in the configuration of the benzhydryl moiety.

Reduction of ketone **1** with sodium borohydride gave a

† See ref 1 and 2 for additional examples of selective reduction of 2-substituted 3-quinuclidinones with aluminum isopropoxide to the cis alcohols.