Synthesis and Biological Activity of Some Antitumor Benzophenanthridinium Salts

Frank R. Stermitz,* John P. Gillespie, Luis G. Amoros, Raquel Romero,† Thomas A. Stermitz,

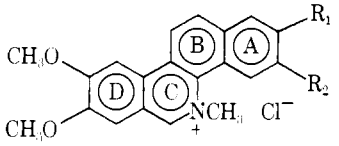
Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523

Kenneth A. Larson, Steven Earl, and James E. Ogg

Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523. *Received February 18, 1975*

A facile synthesis of benzophenanthridinium salts has been developed and used for preparing a number of these compounds. The antitumor activities in mouse leukemia L1210 (LE) and P388 (PS) were determined as well as some selected antimicrobial activities. Although antitumor activity was exhibited by several of the derivatives, none was as active as the naturally occurring alkaloid fagaronine. Fagaronine was made available as a synthetic by an improved procedure. Some structure–activity relationships among antitumor benzophenanthridinium salts are discussed.

Fagaronine (**1a**),[1] isolated from *Fagara zanthoxyloides* (Rutaceae), has shown high activity against L1210 and P388 mouse leukemia but not against B16 melanoma. Nitidine[2] (**1b**), another natural benzo[*c*]phenanthridinium salt,



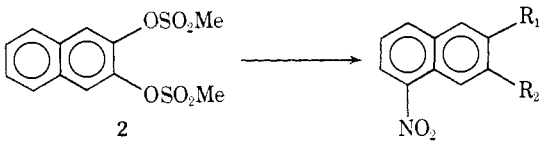
1a, R_1 = OH; R_2 = OCH_3
b, R_1, R_2 = OCH_2O

showed similar, though lower, antileukemic activity in the first two screens but was marginally active against B16 melanoma. In order to establish structure–activity relationships we have prepared a series of substituted benzo[*c*]-phenanthridinium salts using our recently developed synthesis.[3] Structural modifications included omission of one or two methoxy groups, substitution of ethoxy and isopropoxy for methoxy, and altered arrangements of the hydroxy and methoxy functionalities. Also, one isomeric benzo[*a*]-phenanthridinium salt was prepared. We report here the preparation and biological activity of these compounds.

Chemistry. The general procedure utilized has been discussed previously in the report on the synthesis of fagaronine[3] and is shown in Scheme II. Significant improvements have now been made in several steps.

In order to obtain large quantities of 5-nitro-2,3-dimethoxynaphthalene (**3b**),[4] previously available as a minor product in the nitration of 2,3-dimethoxynaphthalene, we sought substituents with a more favorable directive effect (Scheme I). The aromatic mesyloxy group is known to be

Scheme I

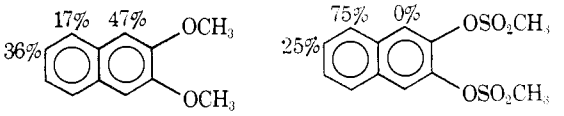


2

3a, R_1 = R_2 = OSO_2Me
b, R_1 = R_2 = OCH_3
c, R_1 = R_2 = OCH_2CH_3
d, R_1 = OH; R_2 = OCH_3
e, R_1 = OCH_3; R_2 = OH
f, R_1 = $OCH(CH_3)_2$; R_2 = OCH_3
g, R_1 = OCH_3; R_2 = $OCH(CH_3)_2$

acid stable but readily removed in base.[5] The NMR of the bismesyloxy derivative **2** revealed that the $H_{1,4}$ protons were shifted downfield 1 ppm relative to 2,3-dimethoxynaphthalene. This was indicative of a strong electron-withdrawing effect of the mesyloxy moities and suggested that nitration might indeed occur in the unsubstituted ring. Although **2** resisted reaction in acetic–nitric acid mixtures, acetyl nitrate[6] effected smooth nitration (see Chart I).

Chart I. Orientation of Nitration in 2,3-Disubstituted Naphthalenes[a]



[a] As determined by liquid chromatography.

Fortuitously, the precipitate which formed as the reaction proceeded was **3a**, uncontaminated by the corresponding 6-nitro isomer which was found to be the minor product. The mesyloxy to methoxy transformation (**3a** → **3b**) was brought about by alternate treatment of a hot aqueous suspension of **3a** with KOH and dimethyl sulfate. In this way **3b** was prepared from 2,3-dihydroxynaphthalene in 40% overall yield—a considerable improvement over previous methods. Subsequent steps as described previously[3] gave the benzophenanthridines **7** (Scheme II). By an analogous route the benzo[*a*]phenanthridine **14** was prepared (Scheme III).

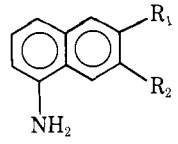
Standard alkylation methods[3] yielded benzophenanthridinium salts (**8**) contaminated by variable quantities of the benzophenanthridines **7** which were extremely difficult to remove by recrystallization. A method of purification of the salts was based upon the well-known[7] addition of alcohols to form the adducts **9**. A chloroform solution of the adduct was then poured onto a plug of silica gel and the adduct **9** was thus converted back to the salt **8**. Further washing removed the unreacted starting material **7**. The salts were then recovered by reconversion to the adduct form. The method failed with the benzo[*a*]phenanthridinium salt **15** where stirring with potassium carbonate and methanol (the usual method for alcohol adduct formation) led to a complex mixture of products. The method was not used with phenolic salts. Hence, these were purified before the isopropoxy group was removed.

Cleavage of the isopropoxy protecting group[8] to yield the phenolic salt was conveniently brought about by heating the appropriate precursor in HBr–HOAc. No cleavage of the NCH_3 or OCH_3 was observed under these conditions. These results are in contrast to the reported[2b] behavior of nitidine salts, which were said to dealkylate readily in water. We have not found N-demethylation to occur under mild conditions in any of our benzophenanthridinium compounds.

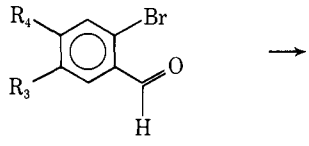
The synthetic method allowed preparation of fagaronine (**1a**) and isofagaronines (**8o–q**) in yields of 5–8% based on

† American Chemical Society Catalyst Program, 1974.

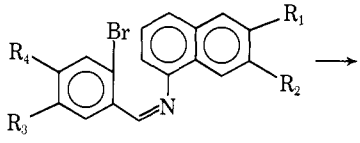
Scheme II



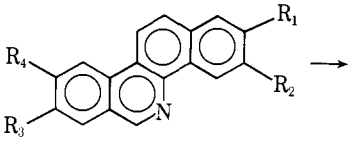
4a, $R_1 = R_2 = OCH_3$
b, $R_1 = R_2 = OCH_2CH_3$
c, $R_1 = OCH(CH_3)_2$; $R_2 = OCH_3$
d, $R_1 = OCH_3$; $R_2 = OCH(CH_3)_2$



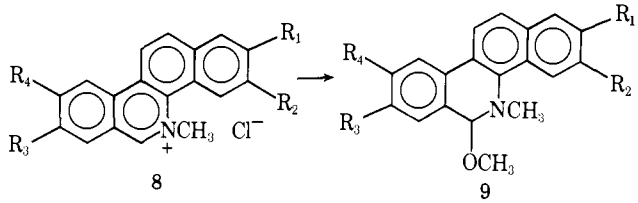
5a, $R_3 = OCH_3$; $R_4 = H$
b, $R_3 = R_4 = OCH_3$
c, $R_3 = OCH_2CH_3$; $R_4 = OCH_3$
d, $R_3 = OCH_3$; $R_4 = OCH_2CH_3$
e, $R_3 = OCH(CH_3)_2$; $R_4 = OCH_3$
f, $R_3 = OCH_3$; $R_4 = OCH(CH_3)_2$



6



7



8 9

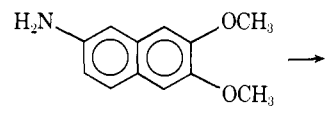
6–9a, $R_3 = R_4 = OCH_3$; $R_1 = R_2 = H$
b, $R_1 = R_2 = OCH_3$; $R_3 = R_4 = H$
c, $R_1 = R_2 = R_4 = OCH_3$; $R_3 = H$
d, $R_1 = R_2 = R_3 = OCH_3$; $R_4 = H$
e, $R_1 = H$; $R_2 = OBz$; $R_3 = R_4 = OCH_3$
f, $R_1 = H$; $R_2 = OH$; $R_3 = R_4 = OCH_3$
g, $R_1 = R_2 = R_3 = R_4 = OCH_3$
h, $R_1 = R_2 = R_3 = OCH_3$; $R_4 = OCH_2CH_3$
i, $R_1 = R_2 = R_4 = OCH_3$; $R_3 = OCH_2CH_3$
j, $R_1 = R_2 = OCH_2CH_3$; $R_3 = R_4 = OCH_3$
k, $R_2 = R_3 = R_4 = OCH_3$; $R_1 = OCH(CH_3)_2$
l, $R_1 = R_3 = R_4 = OCH_3$; $R_2 = OCH(CH_3)_2$
m, $R_1 = R_2 = R_4 = OCH_3$; $R_3 = OCH(CH_3)_2$
n, $R_1 = R_2 = R_3 = OCH_3$; $R_4 = OCH(CH_3)_2$
o, $R_1 = R_3 = R_4 = OCH_3$; $R_2 = OH$
p, $R_1 = R_2 = R_4 = OCH_3$; $R_3 = OH$
q, $R_1 = R_2 = R_3 = OCH_3$; $R_4 = OH$

2,3-dihydroxynaphthalene and taking into account recovered starting materials. Where protecting groups were not necessary, as in the case of the tetramethoxy derivative **8g**, yields were on the order of 10–12% overall. The only low-yield transformations in the syntheses were the cyclization reactions (**6** → **7**). We made no attempt to increase the observed yields of 25–30%. Methods of increasing cyclization yields have been reported recently by Kessar.[9,10]

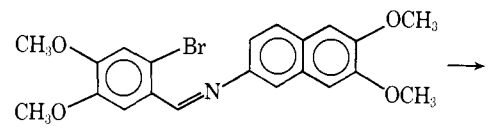
Biological Activity and Discussion. The test results against L1210 and P388 mouse leukemias for the benzophenanthridinium compounds synthesized are given in Table I. The synthetic fagaronine (**1a**) should, of course, yield results identical with the natural material. Our L1210 T/C values (146 and 162) are quite close to those communicated to us through J. L. Hartwell (National Cancer Institute) from the NCI contractors (T/C = 161). Our P388 value (T/C = 190 at 50 mg/kg) is not directly comparable because of different dosages (T/C = 290 at 320 mg/kg by NCI contractors). A further check on our testing procedures is provided by the tetramethoxy compound **8q**. While our work was in progress, this compound was independently synthesized[11] and the following T/C values were reported:[12] L1210 = 131, 134, and 151 at 50, 100, and 200 mg/kg, respectively, and P388 = 165 at 50 mg/kg. Our results on **8q** (Table I) correspond very closely to these values and, hence, we are confident that our bioassay procedure is a valid one for antitumor activity. The results on compounds **8b,d,j,k** were limited because of toxicity and the maximum doses listed in Table I show the beginnings of toxicity in the P388 screens.

In terms of analyzing the activities represented in Table I, it is convenient to group the compounds using the T/C = 125 values as a measurement of borderline activity. This has been adopted by the National Cancer Institute as a general guideline. Four compounds (**1a** and **8c,g,o**) showed values greater than T/C = 125 in both screens, five additional (**8h–l**) gave results greater than 125 in the P388 (but not L1210) screen, and eight (**8a,b,d–f,p,q** and **15**) can be considered inactive. The most striking general result is probably that any variation from the substituent patterns of the three previously known active materials (**1a,b** and **8g**) has caused a decrease in activity. Thus, replacing two of the methoxy groups of the highly active **8g** with ethoxy groups has created a barely active and toxic compound **8j**. Even replacement of one methoxy by an ethoxy (to give **8i**, for example) has greatly decreased activity. Another marked effect of a minor change is reflected in the isomers of fagaronine. Thus, **8o** retains some of the high activity of fagaronine, but the phenolic isomers **8p** and **8q** are inactive. Perhaps the most useful generalization which can be made reflects the fact that the *highly* active benzophenanthridinium salts (**1a,b** and **8g,o**) all have the same dimethoxy substituent pattern at R_3 and R_4 while they vary in substituents at R_1 and R_2. We would therefore suggest that

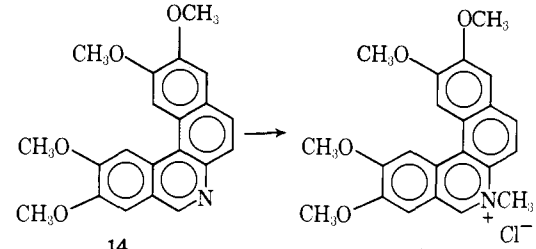
Scheme III



12



13



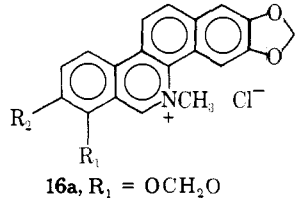
14 15

Table I. Properties and Antileukemic Activity of Benzophenanthridinium Salts[a]

Compd[b]	Formula	Mp, °C	Antileukemic activity		
			Dose, mg/kg	L1210, % T/C (survival)	P388, % T/C (survival)
1a			50	146 (8/8)	190 (8/8)
			100	162 (8/8)	
8a	$C_{20}H_{18}ClNO_2$	235 dec	100	117 (7/8)	98 (7/7)
			150	115 (8/8)	
8b	$C_{20}H_{18}ClNO_2 \cdot H_2O$	176–178	50	109 (7/7)	104 (7/7)
			75	96 (7/7)	78 (6/7)
8c	$C_{21}H_{20}ClNO_3 \cdot 0.5H_2O$	202–203	50	118 (7/7)	130 (7/7)
			100	131 (7/8)	120 (7/7)
			125	111 (7/7)	
			150	117 (4/8)	
8d	$C_{21}H_{20}ClNO_3$	217–220	100	111 (8/8)	124 (7/7)
			200	100 (6/7)	85 (6/7)
8e	$C_{27}H_{24}ClNO_3$	205–206	100	103 (7/8)	
8f	$C_{20}H_{18}ClNO_3 \cdot H_2O$	243–245	50	118 (7/8)	
			100	113 (8/8)	
8g[c]			50	130 (7/8)	153 (24/24)
			100	145 (8/8)	
			150	151 (7/8)	
8h	$C_{23}H_{24}ClNO_4$	269–270	50	119 (8/8)	142 (8/8)
			100	119 (8/8)	152 (8/8)
8i	$C_{23}H_{24}ClNO_4 \cdot H_2O$	290–292	50	114 (7/7)	127 (7/7)
			150	121 (7/7)	135 (7/7)
8j	$C_{24}H_{26}ClNO_4$	269–271	50	113 (7/7)	128 (8/8)
			75	111 (7/7)	75 (5/7)
8k	$C_{24}H_{26}ClNO_4 \cdot H_2O$	265–267	50		121 (7/8)
			75		131 (8/8)
			100		122 (7/8)
8l	$C_{24}H_{26}ClNO_4 \cdot H_2O$	267–269	50		129 (8/8)
			100		137 (8/8)
8m	$C_{24}H_{26}ClNO_4 \cdot H_2O$	253–255	To be tested		
8n	$C_{24}H_{26}ClNO_4 \cdot H_2O$	279–281	To be tested		
8o	$C_{21}H_{20}ClNO_4 \cdot H_2O$	257–260	50	120 (8/8)	141 (8/8)
			75		151 (8/8)
			100	123 (8/8)	147 (6/7)
			150	135 (6/7)	160 (7/7)
8p	$C_{21}H_{20}ClNO_4 \cdot H_2O$	202–204	50	111 (7/7)	117 (7/7)
			100	113 (7/7)	120 (7/7)
			200	116 (7/7)	122 (7/7)
8q	$C_{21}H_{20}ClNO_4 \cdot 0.5H_2O$	261–263	50	107 (7/7)	108 (7/7)
			100	103 (7/7)	107 (7/7)
			150	104 (7/7)	109 (7/7)
15	$C_{22}H_{22}ClNO_4 \cdot 1.5H_2O$	197–199 dec	100	112 (7/8)	
			150	103 (7/8)	

[a] All new compounds listed above gave satisfactory C, H, and N analyses. [b] In all cases, X = Cl. [c] The synthesis[11] and antileukemic activity[12] of 8g have recently been reported.

any further molecular modification work should probably concentrate on the A ring rather than the D ring of the 8 compounds. This idea is reinforced by the fact that previous results have shown **16a** and **16b** to be devoid of antitumor activity, although both were cytotoxic against KB cells at low doses.



16a, $R_1 = OCH_2O$
b, $R_1 = R_2 = OCH_3$

Sanguinarine chloride (**16a**) has been shown to be an antifungal,[13] antiprotozoal,[14] antibacteriophagal,[15] and antibacterial[16] agent and chelerythrine (**16b**) shows similar inhibitory behavior. Because of these activities, we tested a number of our synthetics against *Staphylococcus aureus* and *Escherichia coli* along with **16a** and **16b** and the results are summarized in Table II. In some of the cases difficulties were encountered with water solubilities and suspensions were obtained at high concentrations. In such cases, propylene glycol was also used as a diluent with only minor changes in results. It is evident that none of the derivatives approach the activity of **16a** or **16b** against *S. aureus* or the activity of **16a** against *E. coli*, nor do the values for the synthetics themselves represent significant inhibitory action. We have commented previously[17] on possible

Table II. Minimum Inhibitory Concentrations (MIC) for Selected Benzophenanthridinium Chlorides[a]

Compd	*S. aureus*	*E. coli*
1a	112	225
1b	>1250	>1250
8a	156	1250
8c	156	312
8j	78	156
8g	312	625
8o	312	312
16a	10	39
16b	19	156

[a]Expressed in micrograms per milliliter of water and determined by the serial dilution method.

reasons for the dichotomy of activities shown by **1a** and **1b** as opposed to **16a** and **16b**.

Experimental Section

Synthesis. NMR spectra were recorded on either a Varian T-60 or JOEL JNM-MH-100 instrument. A Beckman Acculab 3 was used for determination of ir spectra. Melting points were taken on either a Thomas-Hoover or a Mel-Temp apparatus. Acceptable analyses were obtained for new compounds. Analyses were by Midwest Microlab.

Ir, NMR, and uv spectra of the synthons and analogs reported here are similar to those observed in the synthesis of fagaronine.[3] Owing to the large number of compounds synthesized, this section contains only general experimental procedures and only those which differ significantly from previous work.

2,3-Dimethanesulfonoxynaphthalene (2). A solution of 50 g (0.31 mol) of 2,3-dihydroxynaphthalene in 250 ml of $CHCl_3$ and 95.7 ml of triethylamine was treated dropwise with 48.6 ml (0.43 mol) of methanesulfonyl chloride. After stirring for 2 hr, the white precipitate was collected by filtration and washed with ethanol to yield 94 g (95%) of **2**. Crystallization from ethanol gave an analytical sample: mp 159–160°; NMR (DMSO-d_6) δ 3.53 (s, 2-OSO_2CH_3), 7.80 (A_2B_2 m, 4 H), 8.10 (s, $H_{1,4}$). Anal. ($C_{12}H_{12}O_6S_2$) C, H, S.

5-Nitro-2,3-dimethanesulfonoxynaphthalene (3a). A suspension of 50 g (0.16 mol) of **2** in 465 ml of acetic anhydride was treated with HNO_3 (70%) in a flask fitted with a mechanical stirrer at such a rate as to keep the temperature between 37 and 39°. *Caution:* a cooling bath should be kept under the flask, ready to be raised if necessary, as the temperature should not be allowed to exceed 45°. In one case an exothermic reaction occurred when the solution reached this temperature, and copious amounts of NO_2 were given off.[6] Over a period of 3 hr, approximately 110 ml (1.2 mol) of HNO_3 had been added. At this time, the reaction mixture was cooled to 5° in an ice bath and the precipitate was collected by filtration and washed with ether. The reaction was monitored by liquid chromatography [uv detector, 0.75 in. × 2 ft Corosil column, chloroform–cyclohexane (35/65)]. Analysis of the precipitate showed that it consisted of only one isomer, **3a** (33 g, 57.8%), while analysis of the filtrate revealed that it contained, in addition to small amounts of **2** and **3a**, a substantial quantity of another isomer, probably 6-nitro-2,3-dimethanesulfonoxynaphthalene. The filtrate was not further investigated but discarded while cold. Recrystallization of **3a** from acetonitrile gave an analytical sample: mp 200–201°; NMR (DMSO-d_6) δ 3.58 (s, 2-OSO_2CH_3), 7.76 (t, J = 8 Hz, H_5), 8.26–8.66 (m, 4 H). Anal. ($C_{12}H_{11}NO_8S_2$) C, H, N, S.

5-Nitro-2,3-dimethoxynaphthalene (3b). **3a** (50 g, 0.11 mol) suspended in 1 l. of 2% NaOH was heated at 100° for 1 hr, resulting in a deep red solution. Dropwise addition of dimethyl sulfate to the hot solution slowly caused a color change to yellow and formation of a precipitate. Monitoring of the reaction by liquid chromatography indicated only partial conversion to **3b**. Sodium hydroxide (20 g) was added to the solution and heating was continued for 1 hr. Again, dimethyl sulfate was added until the color changed. Repetition of the above sequence two more times resulted in a yellow precipitate which was collected by filtration and washed first with 5% NaOH and then with water. Drying of the solid and recrystallization from ethyl acetate gave 23.8 g (74%) of **3b**, mp 151–153° (lit.[4] 155°), identical with an authentic sample.

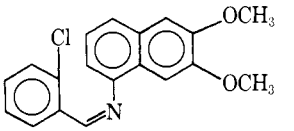
5-Nitro-2,3-diethoxynaphthalene (3c). In analogy with the above procedure, 25 g (0.055 mol) of **3a** was alkylated with diethyl sulfate. However, more repetitions of the hydrolysis–alkylation sequence were required. Extraction of the basic solution with $CHCl_3$, drying, and evaporation of the solvent gave a brownish oil, which crystallized upon addition of ethanol. Recrystallization from the same solvent gave 6.5 g (36%) of **3c**, mp 63–64°, as yellow needles. Anal. ($C_{14}H_{15}NO_4$) C, H, N.

5-Nitro-2-hydroxy-3-methoxynaphthalene (3d), 5-nitro-2-methoxy-3-hydroxynaphthalene (3e), and 5-nitro-2-isopropoxy-3-methoxynaphthalene were prepared as previously reported.[3] In a similar manner, **5-nitro-2-methoxy-3-isopropoxynaphthalene (3g)** was synthesized: 93% yield; mp 104–105° (ethanol). Anal. ($C_{14}H_{15}NO_4$) C, H, N.

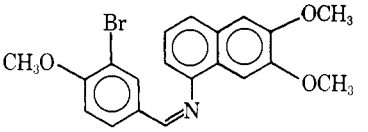
5-Amino-2,3-dimethoxynaphthalene (4a) and 5-amino-2-isopropoxy-3-methoxynaphthalene were obtained from Pd/C and N_2H_4 reduction of the corresponding nitro derivatives as before.[3] Similarly, reduction of **3c** gave **5-amino-2,3-diethoxynaphthalene (4b)**: 89% yield; mp 128–129° (ethanol). Anal. ($C_{14}H_{17}NO_2$) C, H, N. Reduction of **3g** gave **5-amino-2-methoxy-3-isopropoxynaphthalene (4d)**: 99% yield; mp 88–89° (sublimation). Anal. ($C_{14}H_{17}NO_2$) C, H, N. 6-Nitro-2,3-dimethoxynaphthalene gave **6-amino-2,3-dimethoxynaphthalene (12)**: mp 202–203° (sublimation). Anal. ($C_{12}H_{13}NO_2$) C, H, N.

3-Methoxy-6-bromobenzaldehyde (5a),[18] 4-methoxy-3-bromobenzaldehyde,[19] and 3,4-dimethoxy-6-bromobenzaldehyde (5b)[20] were prepared by literature methods. Bromoaldehydes **5c** [mp 115–117° (ethanol). Anal. ($C_{10}H_{11}BrO_3$) C, H], **5d** [mp 110–111° (ethanol). Anal. ($C_{10}H_{11}BrO_3$) C, H], **5e** [mp 72–74° (ethanol). Anal. ($C_{11}H_{13}BrO_3$) C, H], and **5f** [mp 105–107° (ethanol). Anal. ($C_{11}H_{13}BrO_3$) C, H] were prepared by bromination of the corresponding aldehydes in acetic acid in analogy with literature procedures.[20]

Preparation of the anils 6, 10, 11, and 13 was according to the following general procedure. Equimolar amounts of the aldehyde **5**



10



11

and amine **4** were dissolved in benzene (5–10 ml/g of aldehyde) and heated in a 100–110° oil bath in a flask fitted with a Dean-Stark trap. Occasionally, a tiny drop of trifluoroacetic acid was added as a catalyst. The reactions were terminated (when NMR analysis of an aliquot indicated completion by disappearance of the aldehyde proton) by evaporation of the solvent, trituration of the residue with ether, and collection of the yellow solid by filtration. Analytical samples were prepared by recrystallization from ethyl acetate unless otherwise indicated. Physical properties may be found in Table III. Compound **6e** was prepared as follows. A solution of 60 g of the hydroxyanil **6f** in 300 ml of DMF was treated with 60 g of anhydrous K_2CO_3 and 27.9 g of benzyl bromide. After 3 hr of stirring at room temperature, the initial orange color faded and a yellow precipitate formed. The suspension was poured into 2 l. of water and the solid collected by filtration and dried over P_2O_5 to give 65.85 g of **6e**.

Preparation of the benzophenanthridines 7 from the anils 6, 10, and 11 was by the method of Kessar[21] using $NaNH_2$ in liquid ammonia. The physical properties are listed in Table IV. In the case of the cyclization of **13** to **14**, a slight modification of the work-up procedure was necessary. Thus, 15 g (0.035 mol) of **13** was treated with $NaNH_2$ in liquid NH_3. Work-up with chloroform and water and evaporation of the $CHCl_3$ gave a yellow solid. Boiling with ethanol gave 4.1 g of a solid which showed several spots upon TLC analysis. The residue was treated with 100 ml of 33% HCl and extracted with $CHCl_3$. The resulting red emulsion was filtered and the $CHCl_3$ separated and extracted with 10% aqueous KOH. The $CHCl_3$ solution was dried and evaporated leaving 1.2 g of **14** as a tan solid: NMR ($CDCl_3$) δ 4.14 (s, 3-OCH_3), 4.16 (s, -OCH_3), 7.48 (s, $H_{1\text{ or }4}$), 7.54 (s, $H_{1\text{ or }4}$), 8.00 (d, J = 9 Hz, $H_{7\text{ or }8}$), 8.16 (d, J = 9

Table III. Properties of Anils[a]

Compd	Formula	Mp, °C (recrystn solvent)[b]	Yield, %
6a	$C_{19}H_{16}BrNO_2$	166–168	85
10	$C_{19}H_{16}ClNO_2$	143–145 (benzene)	62
11	$C_{20}H_{18}BrNO_3$	155–156	84
6d	$C_{20}H_{18}BrNO_3$	145–147	64
6e	$C_{26}H_{22}BrNO_3$	164–165	89
6f	$C_{19}H_{16}BrNO_3$	188–190 (benzene)	90
6g	$C_{21}H_{20}BrNO_4$	184–185	94
6h	$C_{22}H_{22}BrNO_4$	190	
6i	$C_{22}H_{22}BrNO_4$	165–166	94
6j	$C_{23}H_{24}BrNO_4$	171–172	91
6l	$C_{23}H_{24}BrNO_4$	162–163	92
6m	$C_{23}H_{24}BrNO_4$	144–145	84
6n	$C_{23}H_{24}BrNO_4$	149–150	69
13	$C_{21}H_{20}BrNO_4$	182–183	95

[a] All compounds listed above gave satisfactory C, H, and N analyses. [b] If other than ethyl acetate.

Table IV. Properties of Benzophenanthridines[a]

Compd	Formula	Mp, °C (recrystn solvent)[b]	Yield, %
7a	$C_{19}H_{15}NO_2$	233–234 (benzene)	24
7b	$C_{19}H_{15}NO_2$	170–172 (benzene)	48
7c	$C_{20}H_{17}NO_3$	200–201 (benzene)	28
7d	$C_{20}H_{17}NO_3$	227–228 (benzene)	44
7e	$C_{26}H_{21}NO_3$	211–212 (benzene)	21
7g		300–305	31
7h	$C_{22}H_{21}NO_4$	269–270	
7i	$C_{22}H_{21}NO_4$	289–291	
7j	$C_{23}H_{23}NO_4$	275–275.5	29
7l	$C_{23}H_{23}NO_4$	277–280	23
7m	$C_{23}H_{23}NO_4$	254–257	
7n	$C_{23}H_{23}NO_4$	277–279	43
14	$C_{21}H_{17}NO_4$	255–256 (benzene)	10

[a] All new compounds listed above gave satisfactory C, H, and N analyses. [b] If other than nitromethane.

Hz, $H_{7\text{ or }8}$), 8.59 (s, $H_{1,12}$), 9.35 (s, H_5); ir ($CHCl_3$) 1621 cm^{-1} (-C=N-).

Benzophenanthridium salts 8 and 15 were synthesized by standard alkylation methods[3] (xylene, nitrobenzene, and dimethyl sulfate, 180°). In many cases, TLC and NMR showed that the alkylation reaction did not go to completion and that the methosulfate salts produced were contaminated with variable quantities of the benzophenanthridines 7. Since this contaminant could not readily be removed by crystallization, the following procedure was developed.

Purification of Benzo[*c*]phenanthridinium Salts (8, R ≠ OH). The crude salt obtained from the alkylation reaction was placed in a flask with methanol (20–40 ml/g) and anhydrous K_2CO_3 (2 g/g). Magnetic stirring at room temperature caused the almost immediate disappearance of the yellow color of the salt. The methanol was removed by evaporation. To the residue was added $CHCl_3$, after which the solution and suspended K_2CO_3 were poured onto a plug of silica gel G or P (usually used for preparing thick-layer plates) in a Büchner sintered glass funnel (10–20 g of silica gel/g of salt). Immediately upon contact with the silica gel, the alkoxy derivative 9 was converted back to the salt 8 and a yellow color occurred in the upper portion of the plug. The plug was washed with $CHCl_3$ until TLC revealed that the impurity 7 was no longer being eluted. The silica gel was then transferred back to the flask and stirred with methanol, and more K_2CO_3 was added if the color did not disappear within 5 min. The methanol was removed by evaporation after which the dry powder was transferred back to the funnel and washed with $CHCl_3$. Evaporation of the $CHCl_3$ solution gave the pure adduct 9. The adduct was suspended in methanol and treated with 1–2 ml/g of 33% HCl. A flocculent yellow precipitate occurred and, after 10 min, ether was added and the salt collected by filtration. In some cases, the salts as obtained by this method gave satisfactory elemental analyses without further purification. Where such purification was needed, the salts were either recrystallized from methanol or converted to the methoxy adducts 9, recrystallized from methanol, and reconverted to the salts 8. Conversion of the benzophenanthridines 7 to the chloride salts 8 was virtually quantitative if calculated on the basis of recovered starting material. Typical conversions in the alkylation process were 75+% except in the case of 7l, where only 55% alkylation occurred. Compound 15 could not be purified by the silica gel method since the action of methanol and potassium carbonate on 15 gave a complex mixture of products. Therefore, 15 was purified by multiple recrystallization from ethanol.

Preparation of Fagaronine and Isofagaronines (8o–q). Cleavage of the isopropoxy protecting was accomplished by suspending the appropriate benzophenanthridinium salt in a mixture of acetic acid (70 ml/g) and 48% of hydrobromic acid (5 ml/g) and heating the mixture for 3 hr (8p required 6 hr). The HOAc–HBr mixture was then evaporated, leaving a residue which was triturated with ether and collected by filtration. The resulting bromide salt was then suspended in 240 ml/g of 10% NaCl[2b] and stirred at room temperature for 1 hr. Filtration yielded the chloride salts which were then recrystallized from methanol or methanol–ethyl acetate. Yields varied from 70 to 93%.

Pharmacological Testing. In vivo activity was measured in the P388 and L1210 leukemias according to established procedures.[22] The L1210 cells were obtained from Microbiological Associates, Inc. The P388 cells were obtained from the same source through the courtesy of Dr. G. Sharma of the American Medical Center at Denver.

Quantification of the antimicrobial action was done by measuring the smallest amount of an agent needed to inhibit growth or to kill a test bacterial organism. The minimum inhibitory concentration (MIC) of a test compound was determined by the tube dilution technique using *S. aureus* and *E. coli* from stock cultures maintained in the Department of Microbiology, Colorado State University. A series of culture tubes each containing a medium with a different concentration of an agent was inoculated with the test organism. The tubes were incubated at 37° for 18–24 hr and the lowest concentration of agent that prevented the appearance of turbidity was recorded as the MIC.

Acknowledgment. This work was supported by U.S. Public Health Service Research Grant CA-13648 from the National Cancer Institute. We thank Dr. J. L. Hartwell for a sample of nitidine chloride and for NCI results on antitumor testing of natural fagaronine. The authors thank Larry D. Johnson and Wyatt J. Mills for preparation of some of the intermediates.

References and Notes

(1) W. M. Messmer, M. Tin-Wa, H. H. S. Fong, C. Bellvelle, N. R. Farnsworth, D. J. Abraham, and J. Trojanek, *J. Pharm. Sci.*, 61, 1858 (1972).
(2) (a) M. E. Wall, M. C. Wani, and H. L. Taylor, 162nd National Meeting of the American Chemical Society, Washington, D.C., 1971, Abstracts, MEDI 34; (b) K. Y. Zee Cheng and C. C. Cheng, *J. Heterocycl. Chem.*, 10, 85 (1973).
(3) J. P. Gillespie, L. G. Amoros, and F. R. Stermitz, *J. Org. Chem.*, **39**, 3239 (1974).
(4) C. W. Chang, R. E. Moore, and P. J. Scheuer, *J. Chem. Soc. C*, 840 (1967).
(5) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis", Wiley, New York, N.Y., 1968, p 664.
(6) See ref 5, p 13. Care should be taken, as acetyl nitrate is mentioned as being potentially explosive.
(7) M. Shamma, "The Isoquinoline Alkaloids, Chemistry and Pharmacology", Academic Press, New York, N.Y., 1972, Chapter 17.
(8) T. H. Simpson, *J. Org. Chem.*, **28**, 2107 (1963).
(9) S. V. Kessar, B. S. Phillon, and G. S. Joshi, *Indian J. Chem.*, 11, 624 (1973).
(10) S. V. Kessar, N. Parkash, and G. S. Joshi, *J. Chem. Soc., Per-*

kin Trans. 1, 1158 (1973).
(11) R. K.-Y. Zee-Cheng and C. C. Cheng, J. Heterocycl. Chem., 10, 867 (1973).
(12) R. K.-Y. Zee-Cheng and C. C. Cheng, J. Med. Chem., 18, 66 (1975). We thank the authors for a preprint of their work.
(13) S. A. Vichkanova and V. V. Adgina, Antibiotiki (Moscow), 16, 609 (1971) [Chem. Abstr., 75, 95744 (1971)]; R. D. Stipanovic and A. A. Bell, Pestic. Biochem. Physiol., 2, 364 (1972).
(14) T. Bodalski and H. Rzadkowska, Diss. Pharm., 9, 266 (1957).
(15) T. Bodalski, M. Kantoch, and H. Rzadkowska, Diss. Pharm., 9, 273 (1957).
(16) S. A. Vichkanova, M. A. Rubinchik, V. V. Adgina, and T. S. Fedovchenko, Farmakol. Toksikol. (Moscow), 32, 325 (1969) [Chem. Abstr., 71, 59405e (1969)].
(17) F. R. Stermitz, K. A. Larson, and D. K. Kim, J. Med. Chem., 16, 939 (1973).
(18) H. Hodgson and H. Beard, J. Chem. Soc., 127, 875 (1925).
(19) G. W. Gray, B. Jones, and F. Manson, J. Chem. Soc., 1417 (1956).
(20) R. Pschorr, Justus Liebigs Ann. Chem., 391, 32 (1912).
(21) S. V. Kessar, D. Pal, and M. Singh, Tetrahedron, 29, 169 (1974).
(22) "Protocols for Screening Chemical Agents and Natural Products against Animal Tumors and Other Biological Systems", Drug Evaluation Branch, National Cancer Institute, Silver Spring, Md. 1971.

Biologically Active Polycycloalkanes. 1. Antiviral Adamantane Derivatives

Koji Aigami, Yoshiaki Inamoto,* Naotake Takaishi, Kenichi Hattori,

Industrial Research Laboratories, Kao Soap Company, Ltd., Wakayama 640-91, Japan

Akira Takatsuki, and Gakuzo Tamura

Department of Agricultural Chemistry, The University of Tokyo, Tokyo 113, Japan. *Received January 27, 1975*

Convenient methods for the synthesis of 1-substituted 3-adamantyl chlorides and bromides (**2**), 1-adamantylphenols and -cresols (**3**), and 1-adamantylacetic (**6**) as well as 1,3-adamantanediacetic (**11**) acids are described. Several novel derivatives were synthesized from these key intermediates: adamantylcyclohexanols (**4**) and -cyclohexanones (**5**) from adamantylphenols (**3**), and esters (**7**, **12**, and **22**), amides (**13** and **18**), thioamides (**9** and **16**), amidine (**10**), nitrile (**15**), and amines (**14** and **17**) from 1-adamantanecarboxylic (**19**) and -acetic (**6**) acids and 1,3-adamantanediacetic acid (**11**). Some adamantylpyrimidines (**24**) and -purines (**25** and **26**) were also prepared. Antiviral activities of the compounds obtained in this work and a series of new 1-adamantyl alkyl ketones synthesized before, together with those of some known adamantane derivatives, were tested in vitro on monolayer culture of chick embryo fibroblasts against Newcastle disease virus.

A number of examples have been documented of the use of adamantane compounds as medicines and drugs.[1] However, it seems that many of the compounds tested so far, except for some aminoadamantanes, have been limited to those derivatives which have functional groups of well-known biological activities, and which, therefore, should be regarded as adamantyl analogs of the corresponding drugs. We have prepared various new derivatives of adamantane such as halides, alcohols, ketones, carboxylic acids, esters, amides, nitriles, etc., and have found many of them active as antiviral agents. It is interesting to note that some of the compounds synthesized in this work are more active than amantadine (1-aminoadamantane);[2] although most of them have no particular functional group with established biological activity.

We have recently published preliminary accounts of convenient methods for the synthesis of 1-substituted 3-adamantyl halides,[3] *o*- and *p*-(1-adamantyl)phenols and -cresols,[4] 1-adamantylacetic acid,[5] and 1,3-adamantanediacetic acid.[5] A series of 1-adamantyl alkyl ketones were also prepared.[6] These compounds have served as key intermediates for the synthesis of several derivatives, as summarized in Scheme I.

Chemistry. Ionic[7a] reaction of adamantane with liquid bromine under reflux,[7b] or better at room temperature,[8] offers an excellent method for the preparation of 1-bromoadamantane (**2**, Y = H; X = Br). However, pure 1-chloroadamantane (**2**, Y = H; X = Cl) has been rather difficult to obtain, because of the contamination with 2-chloroadamantane or 1,3-dichloroadamantane (**2**, X = Y = Cl). 2-Chloroadamantane was a by-product in photochlorination[9] of **1**, while 1,3-dichloroadamantane was an impurity in chlorination of **1** by metal chlorides[10] or by *tert*-butyl chloride under aluminum chloride catalysis.[11] We found[3] that pure 1-haloadamantane (**2**, Y = H) free from any contaminating 2-halo and/or 1,3-dihalo compounds was obtained by the reaction of 1-adamantyl cation with halide anion in concentrated sulfuric acid. 1-Adamantyl cation was generated in situ from adamantane by hydride exchange with the *tert*-butyl cation which, in turn, could be formed from *tert*-butyl alcohol in sulfuric acid.[12] The source of halide anion may be alkali metal and alkaline earth metal halides, or hydrogen halides. The method was effective only for the preparation of adamantyl chloride and bromide. Alkali metal fluorides were unreactive toward 1-adamantyl cation, and iodide anion was oxidized to iodine under these reaction conditions.

The halogenation with halide anion in sulfuric acid was applicable to some 1-substituted adamantanes **1** to give the corresponding 3-halo derivatives **2**. This halogenation method was successful, however, only with those compounds which had sufficiently electropositive substituents, in accordance with the carbonium ion nature of the reaction. Taft's polar substituent constant (σ^*)[13] is a measure for the effect of the substituent Y, those having σ^* larger than +1.9 (Y = $CHCl_2$, COOH, Cl, etc.) giving no **2**. This is also consistent with the absence of any 1,3-dihalides in the reaction of unsubstituted adamantane.

1-Phenyl-, 1-methoxy-, and 1-sulfhydryladamantanes gave only 1-haloadamantanes (**2**, Y = H) on reaction with halide anion in sulfuric acid in the presence of *tert*-butyl cation. Cleavage of halo, hydroxy, acetoxy, methoxy,[14-16] and alkylthio[17] groups in the 1 position of adamantane under carbocation-forming conditions is well known, but no example of phenyl group cleavage seems to have been reported. It is interesting that substitution by the *p*-nitro