pounds differed in their fluorescence spectra. When activated at 330 mμ, the N-monoethyl derivative[1] **2** showed a fluorescence maximum at 405 mμ, compound[1] **3** at 390 mμ, derivative **4** at 415 and 455 mμ, and the 6,7-dihydroxy derivative[1] **7** at 410 mμ.

Oral administration of 10 mg/kg (capsules) to four dogs[2] yielded peak plasma concentrations[3] within 0.5–2 hr of about 4 μg/ml. These concentrations decreased with rapid but variable rates with a half-life approximating 1 hr. Blood pressure determinations suggested that a drug plasma concentration of about 0.3 μg/ml had to be reached before a hypotensive response could be detected. However this effect persisted even after the level had dropped below 0.3 μg/ml.

The rapid decrease of blood plasma concentration *in vivo* could be due to rapid excretion, metabolism, or deposition of drug in other body compartments. Very little unchanged drug was found in urine. To distinguish between rapid metabolism and deposition of drug in tissues, five rats were dosed intravenously with **1**. After certain time intervals the animals were sacrificed, plasma was collected, and the total carcasses were homogenized and assayed for total body contents of drug (Table I). Although drug disappeared from the total bodies somewhat more slowly than from plasma, the decrease of drug plasma concentrations seemed to be mainly due to metabolic degradation rather than deposition of drug in other body compartments.

TABLE I

DRUG CONCENTRATIONS IN PLASMA AND TOTAL DRUG IN CARCASSES OF RATS (140 G) AFTER 1.4 mg iv

Rat	Time, hr	Drug plasma concn, μg/ml	Total drug found in carcasses, μg
1	1	5.6	360
2	1	3.3	219
4	2	0.9	107
5	4	0.21	93.3
6	4	0.10	59.5

2-Diethylamino-6,7-dimethoxy-4(3H)-quinazolinone (**1**) was administered orally to humans in two dosage forms. Peak drug plasma concentrations were obtained with capsules after about 2 hr, but with a syrup within 0.5–1 hr. Maximum blood concentrations again were very variable. The highest found was 2 μg/ml after a dose of 300 mg of syrup. Drug plasma half-life was shorter than had been found in dogs, approximating 0.5 hr or even less. As had been the case in the dog studies, it appeared that drug plasma concentration in humans generally also had to reach about 0.3 μg/ml before a decrease in blood pressure occurred.

The search for drug metabolites in human urine yielded five derivatives, **2–6**, besides the parent drug. The sum of these metabolites found in a 24-hr urine sample was estimated to constitute less than 10% of the administered dose.

Metabolite **2** was the only drug derivative found after *in vitro* experiments with rat liver homogenates.

(2) This experiment was carried out in collaboration with Dr. A. Scriabine.

(3) The canine plasma protein binding of the drug (**1**) as determined with Toribara tubes (T. Y. Toribara, *Anal. Chem.*, **25**, 1286 (1953)) was found to be 61% at a concentration of 2 μg/ml.

Experimental Section

Assay of 2-Diethylamino-6,7-dimethoxy-4(3H)-quinazolinone (1).—Plasma (10 ml) was adjusted to pH 9.5–10 by addition of aqueous NaOH and extracted three times with benzene (15 ml each). The benzene solution was concentrated to 5 ml and extracted twice with 0.1 N HCl (4 ml each). The aqueous extract was diluted to 10 ml for fluorometry (Aminco-Bowman). This assay was capable of detecting 0.03 μg of drug/ml of plasma and responded linearly to concentrations between 0.03 and 3 μg/ml. The fluorescence of extracts of plasma from medicated animals was that of unchanged drug.

Carcasses were homogenized with 0.1 N HCl (300 ml) in a Waring Blendor. The homogenate was centrifuged, the supernatant was collected, and the residue was rehomogenized with 0.1 N HCl (300 ml). After centrifugation, the combined supernatants were adjusted to pH 10 with 30% NaOH and the drug was extracted similarly to the procedures described above.

Identification of Metabolites.—Isolation and purification of metabolites followed conventional routes of extraction and thin layer chromatography (tlc). The tlc systems employed silica gel HF and solvent mixtures of either benzene-acetone-acetic acid (10:10:1) or ethyl acetate-diethylamine (19:1).

Metabolite **2** was identified by comparison of its tlc mobilities, fluorescence, and ultraviolet curves with those of an authentic sample.[1] Metabolite **3** was identical with an authentic sample[1] by the same criterion as well as by its infrared absorption spectrum. Metabolite **4** had the same tlc mobility as a monodemethyl derivative of **1** obtained by treatment of **1** with hydrobromic acid. Metabolite **5** appeared to be an isomer of **4** and was converted to drug (tlc) by treatment with diazomethane. The structure **6** is tentative since a conversion of **6** to **2** with diazomethane was not proven unambiguously.

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Carboranes. III. Boron-Containing Acridines[1]

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In a continuation of work designed to synthesize biologically active carboranes[3] and to incorporate a carrier into a carborane molecule which would promote its localization into tumors for neutron-capture therapy, acridines have been considered. As a basis for this work certain acridines have selectively localized in tumor nuclei under *in vivo* conditions[4] intercalcating with the nucleic acids[5] and recently nitrogen mustard containing acridines have shown high antitumor activity.[6] Boron was first incorporated into an acridine

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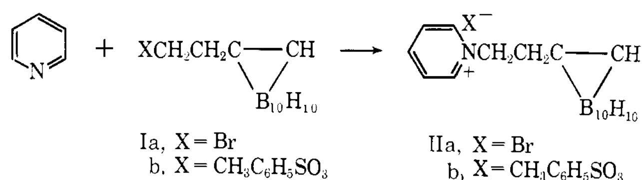
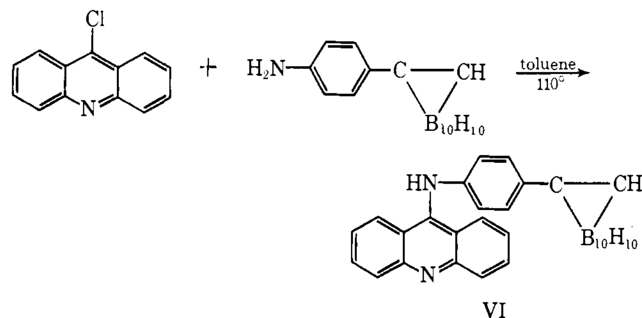
with the synthesis of bis-3,6-(*p*-boronobenzylamino)-acridine,[7] but this compound proved to be highly toxic at doses necessary to attain sufficient boron levels in tissues.[8] On this basis the synthesis of an acridine containing a carborane moiety was undertaken in order to achieve a high percentage of boron in such a molecule.

Chemistry.—Preparations of a carboranylacridine by alkylation of the ring nitrogen of acridine, 9-aminoacridine, 3,6-diaminoacridine (proflavine), and 9-acetamidoacridine with substituted derivatives of carborane [$(B_{10}H_{10}C_2H)CH_2CH_2X$, X = Br, I, and $CH_3C_6H_5SO_3$] and with analogous acetylenic derivatives, which could be converted to the carboranes following alkylation, were singularly unsuccessful. This failure can be largely attributed to steric factors at the 10 position (ring nitrogen) of the acridine molecule.[9] While pyridine condenses readily with bromoethyl- and tosylethylcarborane to give the corresponding salts, 2,6-lutidine (2,6-dimethylpyridine), having a steric arrangement about the ring nitrogen comparable to the acridines, failed to react even at temperatures up to 180°.



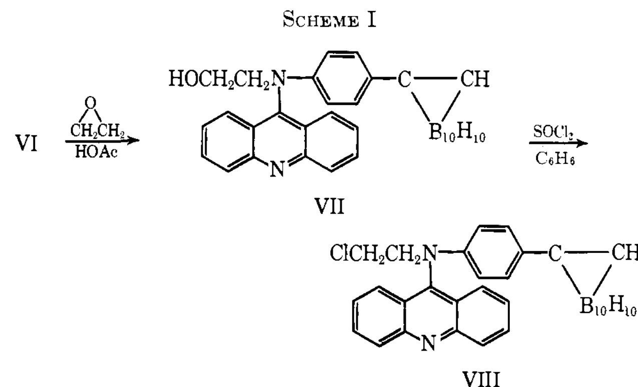
Similarly, acylation of acridines such as 9-aminoacridine, 9-acetamidoacridine, and 9-carbobenzyloxyamidoacridine (III) with carboranyl acid chlorides of the type $(B_{10}H_{10}C_2R)COCl$ [R = C_6H_5 (IVa) and CH_3-CH=CH (IVb)] and with the acetylenic acid chlorides, $C_6H_5C{\equiv}CCOCl$ (Va) and $CH_3CH_2C{\equiv}CCH_2COCl$ (Vb), have proved unsuccessful to date.

An alternative method, which has been widely used in the synthesis of antimalarials of the quinacrine type[10] has resulted in the synthesis of 9-(*p*-carboranyl)-anilinoacridine (VI). Condensation of 9-chloroacridine with *p*-aminophenylcarborane[11] was effected in refluxing toluene, yielding the hydrochloride of VI. Following neutralization with ammonium hydroxide VI was isolated and its carborane structure was confirmed in part by the characteristic B–H absorption band at 2600 cm^{-1} and the carboranyl C–H band at 3100 cm^{-1} in the infrared spectra. The presence of the acridine moiety was confirmed in part by the ultraviolet spectrum which proved to be identical with that of 9-anilinoacridine[12] (λ_{max}^{EtOH} 2450, 2650, 3415, 3580 A).



In view of the observations of Creech, *et al.*,[6] that "one-armed" nitrogen mustards of the quinacrine type exhibit high antitumor activity, attempts were made to synthesize N,N-(2-chloroethyl-*p*-carboranylphenyl)-9-aminoacridine (VIII) by Scheme I.

SCHEME I



Compound VII was synthesized and characterized by infrared and elemental analysis, but all attempts to prepare the "one-armed" nitrogen mustard (VIII) have been unsuccessful to date. Although the structure of the isolated product has not been completely elucidated, the infrared spectra exhibit a split B–H band at 2500 and 2600 cm^{-1} which is characteristic of degradation of the carborane moiety.[13] A possible alternate approach to enhanced biological activity would be the synthesis of the methanesulfonyl derivative (mesylate) of VII, and efforts in this direction are currently under way in our laboratory.

Biological Results.—The single-dose LD_{50}'s of VI and VII were determined by suspending their hydrochlorides in saline containing a few drops of a wetting agent (Tween 40). These suspensions were administered to 6-week-old male CD1 Swiss Albino mice by intraperitoneal injection. The LD_{50} determined in this manner using regression analysis was 200 mg/kg and 180 mg/kg, respectively. These values are comparable to many substituted acridines[14] (*i.e.*, quinacrine, LD_{50} = 280 mg/kg). Thus, on the basis of boron concentration administered, use of these compounds for neutron-capture therapy would appear to be distinctly possible.

The compounds were evaluated for tumor localization by three daily intraperitoneal injections of 18 μg of boron/g into C3H mice bearing subcutaneously transplanted ependymomas.[15] The animals were sacrificed 2 days following the last injection and tissues were analyzed for boron content.[16] The results are summarized in Table I. Both compounds exhibited ele-

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(12) 9-Anilinoacridine was prepared in this laboratory according to the procedure in ref 10; mp 230°, lit.[9] mp 230°.

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TABLE I
TISSUE DISTRIBUTION RATIO[a]

Tissue	VI	VII
Tumor:brain	1.7 (1.3–2.3)	1.1 (0.6–1.4)
Tumor:blood	1.5 (1.0–2.4)	1.3 (0.9–1.7)
Tumor:muscle	0.6 (0.3–0.9)	0.3 (0.1–0.7)
Tumor:liver	<0.1	<0.1
Tumor:spleen	<0.1	<0.1
Tumor:kidney	<0.1	<0.1

[a] Values are averages of nine animals for VI and five animals for VII. The range of values is shown in parentheses.

vated levels in tumor compared with normal brain and blood but localization of the compound did occur in muscle and in other organs such as liver, kidney, and spleen.

In attempts to achieve higher concentrations in tumor with VI, smaller doses were administered over a longer time interval (15 μg of B/g/day for 5 days). Tissue ratios were 2.5 for tumor:brain and 4.2 for tumor:blood. However, at this level an LD_{75} was approached and precluded the use of this compound for neutron-capture therapy.

Interestingly enough, VII showed reduced tumor localization compared with VI and this may be attributable to the requirement of a hydrogen atom on the 9-amino function of quinacrine-like acridines.[4]

Experimental Section[17]

The following compounds were obtained from commercial sources: acridine, 9-chloroacridine, and 3,6-diaminoacridine (K & K Laboratories); 9-aminoacridine (Mann Research Laboratories, Inc.); phenylpropiolic acid (Columbia Organic Chemicals), 3-butyn-1-ol and 3-hexyn-1-ol (Farchan Research Laboratories). The sample of 1-isopropenylcarboranecarboxylic acid was kindly supplied by T. L. Heying and H. Schroeder of the Olin Mathieson Organic Division.

Substituted Acetylenes.—4-Bromo-1-butyne was prepared in 35% yield according to the method of Schlubach, *et al.*,[18] bp 108–110°, lit.[18] 109–110°. 3-Butyn-1-yl toluene-*p*-sulfonate and 4-iodo-1-butyne were prepared in 75 and 65% yields, respectively, according to the procedure of Eglinton and Whiting.[19]

Carboranes.—The carborane derivatives of the acetylenes prepared above were obtained by using the procedure of Fein, *et al.*,[20] bromoethylcarborane, mp 112–114°, lit.[20] 114–115°; tosylethylcarborane (Ib), mp 117–118°; and iodoethylcarborane (Ic), mp 132–134°.

Anal. Calcd for $C_{11}H_{23}B_{10}O_3S$ (Ib): C, 38.60; H, 6.44; B, 31.60; S, 9.36. Found: C, 38.70; H, 6.43; B, 31.51; S, 9.08. Calcd for $C_4H_{15}B_{10}I$ (Ic): C, 16.13; H, 5.40; B, 36.22; I, 42.65. Found: C, 16.25; H, 5.16; B, 36.42; I, 42.40.

Acid Chlorides.—Compounds IVa,b and Va,b were prepared by the action of $SOCl_2$ on the corresponding carboxylic acids. The origin of the acids corresponding to IVb and Va have already been mentioned. The carboxylic acid corresponding to IVa was obtained from the reaction of butyllithium on phenylcarborane followed by addition of CO_2 and hydrolysis.[21] The acid corresponding to Vb was prepared by CrO_3–H_2SO_4 oxidation of 3-hexyn-1-ol.

N-Ethylcarboranylpyridinium Bromide (IIa).—Compound Ia (1.0 g) and 1 ml of pyridine were heated in refluxing benzene for 10 min. The precipitate formed was filtered off and recrystallized from a 1:1 benzene–acetonitrile solution: mp >300°.

Anal. Calcd for $C_9H_{20}B_{10}BrN$: C, 32.74; H, 6.16; B, 32.70; N, 4.31; Br, 24.21. Found: C, 32.76; H, 6.31; B, 32.90; N, 4.49; Br, 24.27.

N-Ethylcarboranylpyridinium Toluene-*p*-sulfonate (IIb).—Compound IIb was prepared in a similar manner to IIa with the exception that external heating was not necessary: mp 255–257°.

Anal. Calcd for $C_{16}H_{27}B_{10}NO_3S$: C, 45.60; H, 6.41; B, 25.62; N, 3.32; S, 7.61. Found: C, 45.45; H, 6.38; B, 25.69; N, 3.50; S, 7.84.

9-Acetamidoacridine was prepared from 9-aminoacridine by both the acetic anhydride method[22] (70%, mp 282°) and the acetyl chloride method (40%, mp 282–283°), lit.[23] mp 266° (uncor).

Anal. Calcd for $C_{15}H_{12}N_2O$: C, 76.55; H, 5.10; N, 11.82. Found: C, 76.75; H, 5.05; N, 12.06.

9-Carbobenzyloxyamidoacridine (III).—9-Aminoacridine (4.0 g) was dissolved in 110 ml of dry dioxane. To this stirred solution was added dropwise 3 ml of carbobenzyloxy chloride and the mixture was allowed to stir overnight at room temperature. The solution was then filtered and the precipitate (9-aminoacridine hydrochloride) was discarded. The filtrate was concentrated under reduced pressure and the obtained solid (0.7 g, 21%) was recrystallized from benzene, mp 212°.

Anal. Calcd for $C_{21}H_{16}N_2O_2$: C, 76.83; H, 4.87; N, 8.53. Found: C, 76.53; H, 4.77; N, 8.32.

9-(*p*-Carboranyl)anilinoacridine (VI).—To a refluxing solution of 0.6 g of 9-chloroacridine in 30 ml of toluene, 0.7 g of *p*-aminophenylcarborane in 10 ml of toluene was added dropwise over 30 min. A precipitate occurred immediately and was filtered off as soon as the addition was complete. The material was washed several times with hot benzene to give 0.7 g (54%) of a yellow crystalline material, mp >300 (presumably the hydrochloride of VI). This material was dissolved in a minimal amount of hot ethanol and made basic to pH 9 with concentrated NH_4OH. Upon addition of H_2O VI precipitated out as a yellow powder, mp 242–243°.

Anal. Calcd for $C_{21}H_{23}B_{10}N_2\cdot HCl$: C, 56.24; H, 5.58; B, 24.13; N, 6.20; Cl, 7.92. Found: C, 56.21; H, 5.64; B, 24.35; N, 6.27; Cl, 7.92.

Anal. Calcd for $C_{21}H_{23}B_{10}N_2$ (VI): C, 61.16; H, 5.82; B, 26.20; N, 6.81. Found: C, 61.29; H, 6.01; B, 26.21; N, 6.59.

N,N-(2-Hydroxyethyl-*p*-carboranylphenyl)-9-aminoacridine (VII).—Compound VI (1.0 g) was dissolved in 10 ml of glacial acetic acid at 10°. To the stirred solution, 5 ml of ethylene oxide was added dropwise over 1 hr. The mixture was then allowed to stir at room temperature for several hours and subsequently poured into ice and water and filtered. The precipitate (1.0 g) was recrystallized from hot benzene (0.5 g, 45%), mp 240° (rearranged at 150°).

Anal. Calcd for $C_{23}H_{28}B_{10}N_2O$: C, 60.53; H, 6.15; B, 23.71; N, 6.15. Found: C, 60.34; H, 6.02; B, 23.36; N, 6.10.

N,N-(2-Chloroethyl-*p*-carboranylphenyl)-9-aminoacridine (VIII).—Attempts to prepare VIII consisted of adding $SOCl_2$ dropwise to refluxing solutions of VII in benzene, $CHCl_3$, and ethyl ether. In all three cases apparent degradation of the carborane moiety occurred. However, when $SOCl_2$ was added to these solutions at room temperature the hydrochloride of VII was obtained.

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(17) Corrected melting points were observed on a Kofler hot stage under a polarizing microscope. The infrared spectra were determined in Nujol or CCl_4 using NaI crystals on a Perkin-Elmer Model 137 spectrophotometer. The ultraviolet spectra were carried out on a Cary Model 11M recording spectrophotometer. Microanalyses were performed by Schwarzkopf Microanalytical Laboratory or by Dr. Stephen M. Nagy, M.I.T. Microanalytical Laboratory.

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