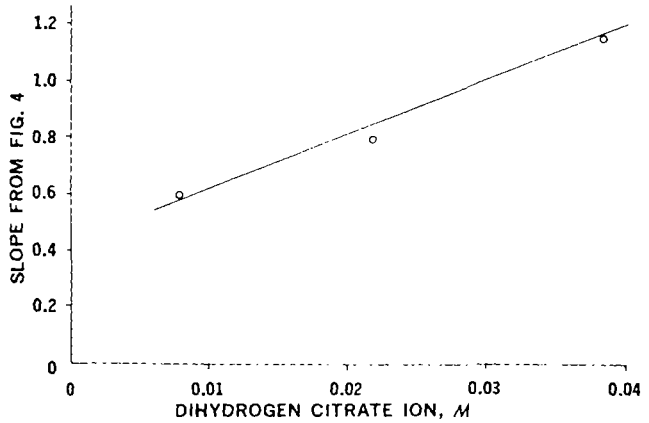


Figure 8—*Effect of dihydrogen citrate ion on the ionic strength effect obtained from the Brønsted–Bjerrum equation in the pH range 4.0–5.0.*

At 45°, pKw is 13.4 (7). Thus, (OH^-) in the pH 7.0 solution is 4×10^{-7}. Using this value and k_0 at pH 7.0 from Table IV, it is possible to calculate that k_{OH^-} is 1.1×10^4 l. mole^{-1} hr.$^{-1}$.

Specific acid-catalyzed hydrolysis, specific base-catalyzed hydrolysis, and monohydrogen citrate-ion-catalyzed hydrolysis all contribute to k_0 at pH 6.5. Thus, k_0 at 45° can be expressed as:

$$k_0 = k_{H^+}(H^+) + k_{OH^-}(OH^-) + k_{C_6H_6O_7^{-2}}[C_6H_6O_7^{-2}] \quad \text{(Eq. 5)}$$

The calculated value of 2.83×10^{-3} hr.$^{-1}$ for k_0 at pH 6.5 also agrees well with the observed value of 4.25×10^{-3} hr.$^{-1}$.

The effect of ionic strength has direct application to the formulation of parenteral and oral liquid penicillin dosage forms. Although it was found that the citrate buffer system provided excellent control over the pH, it contributes to the observed rate of degradation if used below pH 6.5. The buffer system must be selected on the basis of adequate control over pH as well as the absence of catalytic effects.

The ionic strength of the solution has an effect on the chemical stability if a catalytic ion is present. Thus, if a citrate buffer system must be used below pH 6.5, the ionic strength of the solution should be minimized to reduce the catalytic effects of monohydrogen and dihydrogen citrate ions. The minimum effective concentration of such ionic adjuvants as chelating agents, preservatives, and salts used for palatability should be determined to minimize the effect of ionic strength.

REFERENCES

(1) P. Finholt, G. Jurgensen, and H. Kristiansen, *J. Pharm. Sci.*, **54**, 387(1965).

(2) J. P. Hou and J. W. Poole, *ibid.*, **58**, 447(1969).

(3) J. T. Carstensen, *ibid.*, **59**, 1140(1970).

(4) "Geigy Scientific Tables," 6th ed., Geigy Pharmaceuticals, Ardsley, N. Y., 1968, p. 520.

(5) J. W. Poole and C. K. Bahal, *J. Pharm. Sci.*, **57**, 1945(1968).

(6) M. A. Schwartz and F. H. Buckwalter, *ibid.*, **51**, 1119(1962).

(7) "Handbook of Chemistry and Physics," 42nd ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1960, p. 1752.

ACKNOWLEDGMENTS AND ADDRESSES

Received June 28, 1971, from the *Industrial and Physical Pharmacy Department, School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, IN 47907*

Accepted for publication October 29, 1971.

The authors are grateful to Eli Lilly and Co. for generously providing potassium penicillin G.

* Recipient of a Lunsford-Richardson Pharmacy Award, 1971, for a manuscript based on this work.

▲ To whom inquiries should be directed.

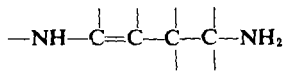
Oxidative Fragmentation of 9-Aminomethylacridan

GEORGE A. DIGENIS*▲ and H. W. WHITLOCK, Jr.†

Abstract □ 9-Aminomethylacridan (*Ia*) and *N,N*-dimethyl-9-aminomethylacridan (IV*a*) were subjected to sodium 1,2-naphthoquinone-4-sulfonate and nitrous acid. Fragmentation of the aminomethyl side chain during oxidation to produce acridine was observed with *Ia* but not with IV*a*. A mechanism based on electrophilic attack at the primary amine nitrogen of the side chain is proposed for the oxidation of *Ia* and similar compounds. The synthesis and purification of the new compounds, *Ia* and its acetamide, IV*a*, 9-hydroxymethylacridan, and the acetamide of 9-aminomethylacridine are described.

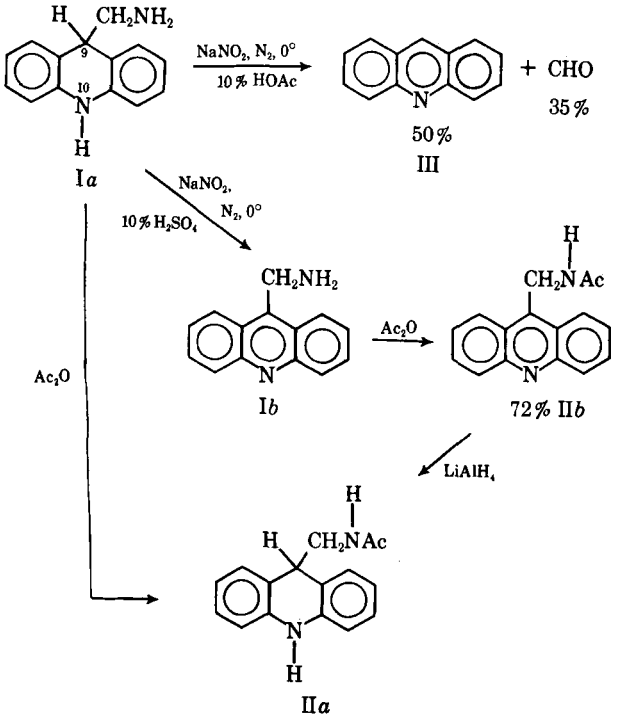
Keyphrases □ 9-Aminomethylacridan—synthesis, oxidative fragmentation, acetamide formation □ *N,N*-Dimethyl-9-aminomethylacridan—synthesis, oxidative fragmentation, acetamide formation □ Acridine—formation by oxidative fragmentation of 9-aminomethylacridan □ Acridans—synthesis, oxidative fragmentation □ Sodium 1,2-naphthoquinone-4-sulfonate—oxidation of acridans □ Nitrous acid—oxidation of acridans □ 9-Hydroxymethylacridan—synthesis

It is believed that the biosynthesis of the alkaloid gramine (IX) from tryptophan involves an oxidative fragmentation of the amino acid's side chain by a pyridoxal-dependent enzyme (1). Since tryptophan and 9-aminomethylacridan[1] (*Ia*) have the common structual unit:



the behavior of *Ia* toward pyridoxal hydrochloride was studied. When *Ia* was treated with pyridoxal hydrochloride, it was found to suffer an oxidative amine

[1] 9,10-Dihydroacridines are referred to as derivatives of the acridan nucleus throughout this paper.



Scheme I

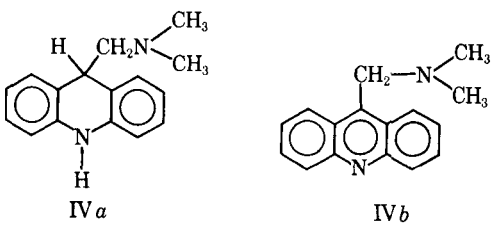
fragmentation (2) to acridine (III). It was recently observed that this reaction could be repeated using nitrous acid or sodium 1,2-naphthoquinone-4-sulfonate (V) instead of pyridoxal. These experiments are described in detail here, and a mechanism is proposed for each reaction.

RESULTS AND DISCUSSION

9-Aminomethylacridan (I*a*),[1] made by lithium aluminum hydride ($LiAlH_4$) reduction of 9-cyanoacridine, was subjected to sodium nitrite ($NaNO_2$) in 10% aqueous H_2SO_4 to give 9-aminomethylacridine (I*b*) in 72% yield (Scheme I). Compound I*b* was isolated in its stable *N*-acetyl form[2] (II*b*). To confirm the product's identity, amide II*b* was reduced with $LiAlH_4$ to give the acetamide of 9-aminomethylacridan (II*a*). Compound II*a* was then synthesized independently by acylation of I*a* (Scheme I) and also by catalytic reduction of 9-cyanoacridine with acetic anhydride and Raney nickel.

When I*a* was treated with $NaNO_2$ in 10% aqueous acetic acid (pH 4) under conditions similar to those of the 10% H_2SO_4 reaction already described, acridine (III) and formaldehyde were isolated in 50 and 35% yields, respectively[3] (Scheme I).

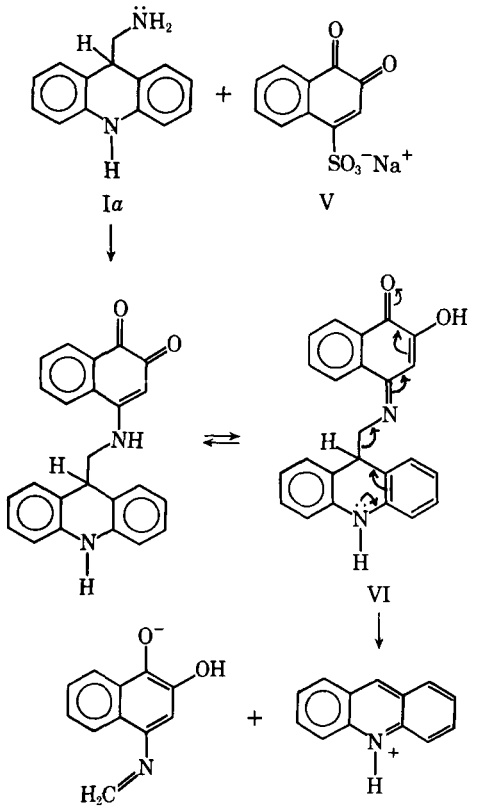
The disparity in the results of the two diazotization reactions could be explained by the fact that at the lower pH the aliphatic nitrogen is protonated and, therefore, is not liable to attack by an electrophilic species (*i.e.*, NO^+). This conclusion is supported by the finding of Kornblum and Iffland (4) that aliphatic primary amines,



IV*a* IV*b*

[2] The synthesis of I*b* was reported previously (3). Because the compound was found to be quite unstable, it was isolated in its stable *N*-acetyl form (II*b*).

[3] In a control experiment where the same reaction was repeated excluding the $NaNO_2$, only starting material (I*a*) was recovered.



Scheme II

such as ethyl, methyl, and propyl amines, do not react with nitrous acid at a pH below 3.

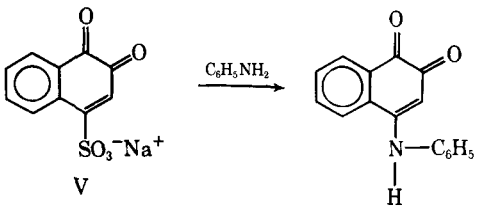
To elucidate further the mechanism of the oxidative fragmentation of I*a*, the amine was treated with sodium 1,2-naphthoquinone-4-sulfonate (V). Compound III and formaldehyde were isolated from this reaction mixture in 54 and 15% yields, respectively. When the same reaction was repeated using the *N,N*-dimethyl-9-aminomethylacridan (IV*a*)[4], it did not yield acridine but Compound IV*b* (in 70% yield).

Because such a diversion in the reaction pathway occurred when the 1° amine function of I*a* was changed to a tertiary amine, it was concluded that the site of attack of I*a* by V was, indeed, the 1° amine function as shown in Scheme II.

The initial attack of I*a* on V probably occurs at the 4-position, whereby the sulfonate moiety is displaced and a Schiff base (VI) is formed. The mechanism of the sulfonate displacement is known to occur with substituted quinones and amines (5), as in the reaction of V with aniline (6) shown in Scheme III.

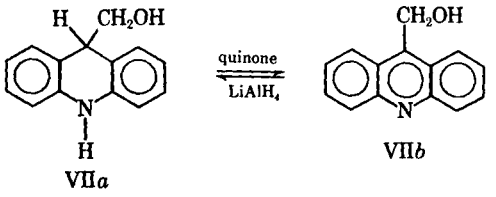
The mechanism proposed in Scheme II is further supported by the fact that when amino acids are treated with pyridoxal or V, carbon dioxide is liberated (7). The formation of a Schiff base intermediate of type VI (Scheme II) followed by electron shifts toward the quinone moiety is believed to be responsible for the observed liberation of CO_2.

The quinone-acridan species (VI) in Scheme II is a Schiff base similar to the pyridoxylidene Schiff base formed between I*a* and



Scheme III

[4] Synthesis described under *Experimental*.



Scheme IV

pyridoxal (2). Furthermore, IV*a* does not undergo fragmentation to produce III either with 1,2-naphthoquinone sulfonate or with pyridoxal hydrochloride.

When V is allowed to react with 9-hydroxymethylacridan (VII*a*)[5], 9-hydroxymethylacridine (VII*b*)[6] and not acridine is formed (Scheme IV).

If the aromatic ring nitrogen had been the site of electrophilic attack, acridine would have been the product of the reaction between IV*a* and pyridoxal or V and of the reaction between VII*a* and V.

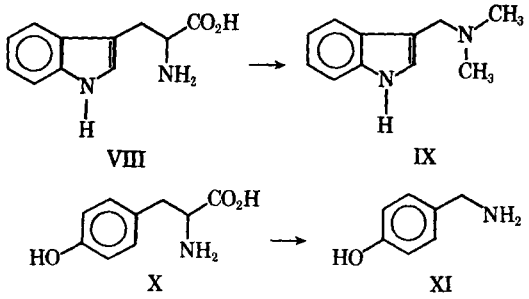
Thus, the results of these experiments tend to support a mechanism based on covalent bond formation between the primary amine nitrogen of I*a* and the electrophilic species (NO^+ or V). Subsequent electronic shifts are followed by oxidative fragmentation to produce III.

In contrast, it was recently observed that the oxidation of 2-chloro-9-[3-(dimethylamino)propyl]acridan[7], the antipsychotic drug, by riboflavin phosphate in the presence of visible light to its acridine derivative, 2-chloro-9-[3-(dimethylamino)propyl]acridine, does not involve covalent bond formation. Rather, it appears to involve triplet–triplet energy transfer by light-excited riboflavin to the dihydroacridine and subsequent hydrogen elimination (9). Furthermore, preliminary results indicate that the riboflavin phosphate-catalyzed oxidation of I*a* also proceeds *via* a triplet–triplet energy transfer reaction. Both I*a* and 2-chloro-9-[3-(dimethylamino)-propyl] acridan were recently found to inhibit electron transport and oxidative phosphorylation in *Pseudomonas saccharophila* (10).

There are several known biotransformations involving fragmentation of amines, two examples being the biosynthesis of gramine (IX) from tryptophan (VIII) (11) and that of *p*-hydroxybenzylamine (XI) from tyrosine (X) (12) (Scheme V). It has been suggested that these biosyntheses involve fragmentation of pyridoxylidene intermediates which are structurally similar to VI. However, on the basis of what is described in this paper, one can suspect that these biosyntheses could be discussed in terms other than pyridoxal-catalyzed reactions.

EXPERIMENTAL[8]

Preparation of 9-Aminomethylacridan (I*a*)[9]—9-Cyanoacridine, m.p. 181–183°, was prepared in 67% yield according to the procedure of Lehmstedt and Dostal (13). A solution of 2.0 g. (0.0098 mole) of 9-cyanoacridine in 250 ml. of dry diethyl ether was added slowly into a previously stirred (for 1.5 hr.) mixture of 3–4 g. (5–10-fold excess) of lithium aluminum hydride ($LiAlH_4$) and 450 ml. of diethyl ether. The mixture was refluxed with stirring under nitrogen for 24 hr. At the end of this time, the excess $LiAlH_4$ was decomposed



Scheme V

by slowly adding a 50% aqueous solution of sodium potassium tartrate to the cooled reaction (0–5°). The aqueous layer of the filtered reaction mixture was extracted with ether and hot benzene. Evaporation of the solvents at 35° under reduced pressure yielded a white crystalline compound, which was purified by two sublimations at 110–120°/0.08 mm. to yield 2.0 g. (0.0095 mole, 97% yield) of I*a*, m.p. 183–185°. Three recrystallizations from methanol afforded the analytical sample as white needles, m.p. 184–185°.

Anal.—Calc. for $C_{14}H_{14}N_2$: C, 79.96; H, 6.71; N, 13.32. Found[10b]: C, 79.90; H, 6.36; N, 13.24.

The IR spectrum showed $\lambda_{max.}^{CHCl_3}$ at 2.81–2.87 (s) broad, 2.93 (m), 6.22 (s), 6.33 (s), 6.80 (v.s.), 7.10 (s), 7.70 (v.s), 8.06(m), 8.68 (m), 11.1 (m) broad, 11.4 (m) broad, and 11.9 (m) μ broad.

The UV spectrum showed $\lambda_{max.}^{MeOH}$ 282 nm. (1.6×10^4).

The NMR spectrum showed a multiplet centered at τ 3.05 (8H) assigned to the aromatic protons, a singlet at τ 3.92 (1H) assigned to the aromatic nitrogen's proton, a triplet at τ 6.1 (1H) (J = 6 Hz.) assigned to the 9-position proton, a doublet at τ 7.2 (2H) (J = 5.5 Hz.) assigned to the methylene protons, and a singlet at τ 9.01 (2H) assigned to the protons of the aliphatic nitrogen.

Preparation of *N,N*-Dimethyl-9-aminomethylacridan (IV*a*)[9]—The method is mainly that of Bowman and Stroud (14). To a solution of 0.370 g. (0.0018 mole) of I*a* (recrystallized from methanol, m.p. 185°) in 30 ml. of methanol and 5 ml. of 40% formaldehyde aqueous solution in a hydrogenation vessel was added 0.5 g. of 10% palladium-on-charcoal.

The mixture was hydrogenated [hydrogen uptake was 89 ml. (0.0036 mole)] at room temperature and atmospheric pressure for 18 hr. The filtrate from the mixture was concentrated to about 5 ml. A white crystalline compound precipitated, which was filtered and dried at room temperature under vacuum for 24 hr. to yield 0.350 g. (83% yield) of IV*a*, m.p. 160–165°, which was recrystallized twice from chloroform–hexane, m.p. 165–167°. Two further recrystallizations from chloroform yielded the analytical sample as white needles, m.p. 166–167°.

Anal.—Calc. for $C_{16}H_{18}N_2$: C, 80.63; H, 7.61; N, 11.75. Found[10a]: C, 81.02; H, 7.28; N, 11.92.

The IR spectrum showed $\lambda_{max.}^{CHCl_3}$ at 2.9 (m), 3.40 (s), 3.6 (m), 6.18 (s), 6.21 (s), 6.3 (m), 6.6 (m), 6.8 (v.s.), 7.1 (m), 7.35 (m), 7.8 (s), 7.7 (m), 8.6 (m), 8.75 (m), 9.15 (m), 9.6 (m), 10.45 (m), 11.0 (s), and 11.7 (s) μ.

The UV spectrum showed $\lambda_{max.}^{MeOH}$ 282 nm. (1.6×10^4).

The NMR spectrum showed a multiplet centered at τ 2.98 (8H) assigned to the aromatic protons, a singlet at τ 3.96 (1H) assigned to the proton of the aromatic nitrogen, a triplet at τ 5.91 (1H) (J = 7.5 Hz.) assigned to the 9-position proton, a doublet at τ 7.61 (2H) (J = 7.5 Hz.) assigned to the methylene protons, and a singlet at τ 7.78 (6H) assigned to the protons of the two methyl groups.

Reaction of 9-Aminomethylacridan (I*a*) with Nitrous Acid in 10% H_2SO_4: Preparation of Acetamides II*a*[9] and II*b*[9]—A solution of 0.7 g. (0.0033 mole) of I*a* in 75 ml. of 10% w/v H_2SO_4 was cooled to 0°, and 0.345 g. (0.005 mole) of sodium nitrite ($NaNO_2$) in 50 ml. of water was added slowly for 1 hr. with stirring under nitrogen. After the reaction was stirred at 0° for 7 hr. more, the excess $NaNO_2$ was decomposed by adding a solution of 0.7 g. of urea in 5.0 ml. of water.

[5] Synthesized according to the procedure of Bergmann and Rabinovitz (8).

[6] As proof of structure, VII*b* was reduced back to VII*a* by $LiAlH_4$ in ether (*Experimental* section).

[7] SKF 14,336.

[8] NMR spectra were measured at 60 mc./sec. (Varian A-60 spectrometer), using deuterated $CHCl_3$ as the solvent and tetramethylsilane as the internal standard (τ 10.0 p.p.m.). Samples were 10% in concentration. UV spectra were recorded on a spectrophotometer (Cary model 11 MS), and IR spectra were recorded on a spectrophotometer (Baird model B). Melting points, determined (Fisher-Johns apparatus) by the hot-block method, were uncorrected.

Thin-layer chromatograms prepared from silica gel G (according to Stahl, E. Merck, Darmstadt, Germany) were of 0.25-mm. thickness. They were activated at 120° for 30 min. and, after spotting, were run ascending in preequilibrated chambers. Spots were first traced with a longwave UV lamp and then developed in an iodine bath. All preparatory thin-layer plates (*i.e.*, more than 0.25 mm. of thickness) were air dried for 12 hr. before activation.

[9] New compound.

[10] Analyses were performed by the following microanalytical laboratories: (*a*) Spang Microanalytical Laboratory, Ann Arbor, Mich.; (*b*) Illini Laboratories, Urbana, Ill.; and (*c*) Schwarzkopf Laboratory, Woodside, N. Y.

The orange-yellow solution was first extracted for acridine with chloroform; however, the combined chloroform extracts, washed with a saturated $NaHCO_3$ solution, dried, and evaporated, left only a minute quantity of material which was discarded.

The aqueous solution was then made basic with 50% NaOH and extracted with chloroform. The combined chloroform extracts were evaporated under reduced pressure to afford 0.8 g. of a thick brown oil, which was immediately added to a stirring mixture of 15 ml. of acetic anhydride and 1.5 g. of anhydrous sodium acetate. After 24 hr. of stirring, 60 ml. of water was added and stirring was continued for 3 hr. The reaction mixture was neutralized with a saturated solution of $NaHCO_3$ and extracted with chloroform. Evaporation of the chloroform at 25° under reduced pressure left 0.668 g. of a crude yellow solid, which was chromatographed on 66 g. of neutral alumina[11]. Elution with chloroform–hexane (1:1) gave 0.596 g. (72.1% yield) of II*b* as a yellow crystalline compound, m.p. 228–232°. Four more recrystallizations from benzene afforded the analytical sample as yellow rhomboid crystals, m.p. 232–234°.

Anal.—Calc. for $C_{16}H_{14}N_2O$: C, 76.78; H, 5.64; N, 11.19. Found[10a]: C, 77.01; H, 5.76; N, 11.30.

The IR spectrum showed $\lambda_{max.}^{CHCl_3}$ at 2.91 (m), 3.3 (m), 6.08 (s), 6.7 (s), 8.2 (s), and 8.4 (s) μ broad.

The UV spectrum showed $\lambda_{max.}^{MeOH}$ 251 and 358 nm. (1.5×10^5 and 1.2×10^4, respectively).

Reduction of the Acetamide of 9-Aminomethylacridine (II*b*) with Lithium Aluminum Hydride—A mixture of 0.081 g. (0.00032 mole) of the acetamide of II*b*, Raney nickel, and 80 ml. of absolute ethanol was hydrogenated at room temperature (19°) and atmospheric pressure for 22 hr. The catalyst was then filtered off, and the filtrate was evaporated to dryness under reduced pressure. The residue was recrystallized three times from benzene, yielding 0.030 g. (37.5% yield) of a white crystalline compound identified as II*a* by m.p. 218–220°, mixed m.p. 216°, and IR and UV spectra comparison with the acetamide of 9-aminomethylacridan (II*a*) prepared below.

Acetamide of 9-Aminomethylacridan (II*a*) from 9-Cyanoacridine—The procedure is mainly that of Gould *et al.* (15). A mixture of 5.0 g. of 9-cyanoacridine (0.0246 mole), 3–4 g. of Raney nickel, and 12 g. of anhydrous sodium acetate in 120 ml. of acetic anhydride was hydrogenated at room temperature (25°) at an initial pressure of 32 p.s.i. (in a Parr hydrogenation apparatus) for 12 hr. After the hydrogenation was completed, the Raney nickel was filtered off the solution; 40 ml. of water was added to the cooled reaction mixture, followed by stirring for at least 3 hr. It was then neutralized with a saturated solution of $NaHCO_3$ and extracted with chloroform. The extracts were dried over anhydrous K_2CO_3 and distilled at 30° under reduced pressure to afford 5.2 g. (84% yield) of II*a* as a white crystalline compound. Four recrystallizations from benzene afforded the analytical sample as long white needles, m.p. 218–220°.

Anal.—Calc. for $C_{16}H_{16}N_2O$: C, 76.16; H, 6.39; N, 11.10. Found[10b]: C, 76.69; H, 6.22; N, 10.94.

The IR spectrum showed $\lambda_{max.}^{mineral\ oil}$ at 2.99 (m), 3.05 (m), 6.06 (s), 6.21 (m), 6.46 (s), 6.72 (s), 7.64 (m), 7.63 (m), 7.78 (m), 8.19 (m), 9.48 (m), 13.2 (m), 13.45 (s), and 15.05 (s) μ.

The UV spectrum showed $\lambda_{max.}^{MeOH}$ 282 nm. (1.6×10^4).

Acetamide of 9-Aminomethylacridan (II*a*) from 9-Aminomethylacridan (I*a*)—A mixture of 1 g. (0.0048 mole) of I*a* and 1 g. of anhydrous sodium acetate in 10 ml. of freshly distilled acetic anhydride was stirred for 16 hr. At the end of this time, it was cooled to 5°; 10 ml. of water was added followed by stirring for 3 hr. more. It was then neutralized with a saturated solution of $NaHCO_3$ and extracted with chloroform. The combined chloroform extracts were dried over anhydrous K_2CO_3 and distilled at 30° under reduced pressure to afford 0.9 g. (74% yield) of II*a* as a white crystalline compound, m.p. 210–220°. Three recrystallizations from benzene afforded white needles, m.p. 218–220°, which were found to be identical [by mixed melting point (218–220°) and IR and UV spectra] to the amide (II*a*) made by the catalytic reduction of 9-cyanoacridine in the presence of Raney nickel and acetic anhydride.

Reaction of 9-Aminomethylacridan (I*a*) with Nitrous Acid in 10% Acetic Acid—To a solution of 0.918 g. (0.0043 mole) of I*a* in 60 ml. of 10% aqueous acetic acid in a two-necked, 100-ml. flask, equipped with a nitrogen inlet and a serum cap, cooled in an ice bath, was added slowly with stirring under nitrogen 1.5 g. (0.0215 mole) of $NaNO_2$ in 5 ml. of water. The reaction mixture was stirred in the ice bath for 4 hr. At the end of this time, the orange-yellow solution was extracted for acridine with chloroform. The combined chloroform extracts were dried over anhydrous K_2CO_3 and evaporated at 25° under reduced pressure. The residue was chromatographed on 50 g. of neutral alumina[11]. Elution with chloroform–hexane (3:7) gave 0.37 g. (50% yield) of III, which was recrystallized twice from hexane to afford yellow needles, m.p. 107–108° (mixed melting point 107–108°). The IR and UV spectra were identical to those of authentic acridine.

Formaldehyde Isolation—The diazotization of I*a* in 10% acetic acid was repeated using solutions of 0.2 g. (0.00095 mole) of I*a* in 20 ml. of 10% acetic acid and 0.0864 g. (0.0012 mole) of $NaNO_2$ in 5 ml. of water. At the end of the reaction time, the mixture was steam distilled under nitrogen into a solution of 0.3 g. (0.0021 mole) of dimedone[12] in 8 ml. of ethanol and 20 ml. of water. With the addition of water to the reaction mixture, 75 ml. of distillate was collected, which was then stirred for 24 hr. The white crystalline precipitate formed was filtered off and recrystallized twice from ethanol–water (1:1) to afford 0.096 g. (35% yield) of methylenebisdimedone, m.p. 189°, which was characterized by mixed melting point (188–189°) and IR spectra comparison with an authentic sample.

Oxidation of 9-Aminomethylacridan (I*a*) by Sodium 1,2-Naphthoquinone-4-sulfonate (V)—In a two-necked, 25-ml. flask, fitted with a nitrogen inlet and a side arm equipped with a serum cap, a solution of 0.101 g. (0.00048 mole) of I*a* in 5 ml. of methanol was introduced. The mixture was cooled to −40° and degassed with a mercury diffusion pump (less than 0.01 mm.) for 1 hr. The system was placed under nitrogen by repeated (at least seven times) evacuation and introduction of nitrogen. A solution of 0.151 g. (0.00058 mole) of V[13] in 6 ml. of water and 5.5 ml. of methanol was quickly introduced by means of a syringe.

The temperature was subsequently adjusted to 0°. Aliquots, withdrawn from the reaction mixture by a syringe through the serum cap at 3 min. and 2.3 and 6 hr. after the addition of the quinone, exhibited spots (R_f 0.25) corresponding to III by TLC[8] on silica gel G[14] (6:4 $CHCl_3$–hexane solvent). UV spectra (in methanol) of these aliquots showed characteristic acridine maxima at 250 and 356 nm. The reaction mixture was stirred in the dark and at 0° for 12 hr. and was subsequently extracted with $CHCl_3$ until the extracts left no residue upon evaporation under reduced pressure.

The combined $CHCl_3$ extracts were filtered through a column of 10 g. of neutral alumina[11] and distilled under reduced pressure. Tha residue (0.049 g.) was then chromatographed on 5 g. of neutrle alumina[11], and 5-ml. fractions were collected. Elution with $CHCl_3$–hexane (2.5:7.5) gave 0.046 g. (54% yield) of III, m.p. 101–104°. Two recrystallizations from hexane yielded pure yellow crystals (m.p. 107–108°), which exhibited identical IR spectra with authentic acridine. The mixed melting point was 107–108°.

Formaldehyde Isolation—The above reaction was repeated using 0.141 g. (0.00066 mole) of I*a* and 0.187 g. (0.00072 mole) of V. To the aqueous layer of the reaction mixture, a solution of 0.3 g. (0.0021 mole) of dimedone in 8 ml. of ethanol was added, and the mixture was stirred for 24 hr. at room temperature. The resulting white crystalline precipitate was filtered off and recrystallized twice from ethanol–water (1:1) to afford 0.029 g. (15% yield) of methylenebisdimedone, m.p. 189°. A mixed melting point (188–189°) and IR spectra proved it to be the formaldehyde–dimedone derivative.

Oxidation of *N,N*-Dimethyl-9-aminomethylacridan (IV*a*) with Sodium 1,2-Naphthoquinone-4-sulfonate (V): Isolation of *N,N*-Dimethyl-9-aminomethylacridine (IV*b*)[9]—A solution of 0.120 g. (0.0005 mole) of IV*a* in 7 ml. of methanol and 0.142 g. (0.00055 mole) of V in 4 ml. of water was allowed to react at room temperature in the dark and under nitrogen for 4.5 hr. as described in the preceding reaction. At the end of this time, the reaction mixture was extracted with $CHCl_3$. The combined $CHCl_3$ extracts were distilled under reduced pressure to afford 0.09 g. of a residue, which was chromatographed on two silica gel G[14] preparatory TLC[8] plates, 20 × 20 cm. × 0.75 mm. (18 g. of silica gel on each), which were first dried at room temperature for 12 hr. and then at 60° for 1 hr. Elution with methanol–$CHCl_3$ (0.5:9.5) yielded a yellow band with R_f 0.5, which was removed from the plate and extracted with methanol and $CHCl_3$. The residue (0.08 g.) from the extracts, which decomposed rapidly on exposure to air, was sublimed twice at 60°/0.02 mm. to afford 0.076 g. (70% yield) of IV*b* as a yellow amor-

[11] Fisher.
[12] Matheson, Coleman and Bell, Norwood, Ohio; m.p. 148°.
[13] Eastman Organic Chemicals, recrystallized according to Follins' method (16).
[14] Merck, Darmstadt, Germany.

phous mass, m.p. 60–70°, which resisted recrystallization from all common solvents.

The IR spectrum showed $\lambda_{max.}^{CHCl_3}$ at 3.42 (s), 3.55 (m), 3.65 (s), 6.5 (m), 6.63 (s), 6.9 (s), 7.4 (m), 8.05 (m) broad, 8.6 (m), 8.75 (m), 8.9 (m), 9.2 (m), 9.98 (s), 11.55 (m), and 11.92 (s) μ.

The UV spectrum was characteristic of an acridine compound: $\lambda_{max.}^{MeOH}$ 250 and 356 nm. (1.5×10^5 and 1.2×10^4, respectively).

A solution of 0.076 g. (0.00035 mole) of the compound in 5 ml. of 95% ethanol was added to 10 ml. of a saturated picric acid solution in 95% ethanol. A yellow crystalline precipitate appeared, which was immediately filtered off the solution and dried; it gave 0.139 g. (86% yield) of the monopicrate of IV*b*, m.p. 170–175° dec. Three more recrystallizations from aqueous ethanol afforded the analytical sample as yellow needles, m.p. 181 183° dec.

Anal.—Calc. for $C_{22}H_{19}N_5O_7$: C, 56.77; H, 4.11; N, 15.05. Found[10a]: C, 56.56; H, 4.15; N, 14.98.

Oxidation of 9-Hydroxymethylacridan (VII*a*) with Sodium 1,2-Naphthoquinone-4-sulfonate (V): Preparation of 9-Hydroxymethylacridine (VII*b*)[9]—Compound VII*a*, m.p. 135–136°, was prepared in 70% yield according to the procedure of Bergmann and Rabinovitz (8). A 10.0-ml. methanolic solution of 0.231 g. (0.0010 mole) of VII*a* in a 50-ml. hydrogenation flask was degassed with a mercury diffusion pump (less than 0.01 mm.) at −40°. Taking care to exclude air, a solution of 0.317 g. (0.00122 mole) of V in 10 ml. of water was introduced through the side arm, and the system was reevacuated for an additional 30 min. After repeated (at least seven times) evacuation and introduction of nitrogen, the reaction mixture was stirred in the dark under nitrogen and at room temperature for 14 hr. At the end of this time, the yellow precipitate formed was filtered off and recrystallized once from chloroform–methanol (1:3) to yield 0.207 g. (90% yield) of VII*b* as yellow needles, m.p. 150–154°. This compound, when chromatographed on a TLC plate[8] on silica gel G[14], had R_f 0.12 with methanol–chloroform (0.15:9.85) and R_f 0.17 with methanol–chloroform (0.25:9.75) eluting solvents; no other spots were detected. Three more recrystallizations from chloroform–methanol (1:3) yielded yellow needles, m.p. 153–155°.

The IR spectrum showed $\lambda_{max.}^{KBr}$ at 2.85 (m) broad, 3.17 (s), 6.18 (m), 6.22 (m), 6.4 (m), 6.6 (m), 6.85 (m), 6.90 (m), 7.4 (m), 8.7 (m), 9.6 (s), 9.95 (v.s.), 13.1 (s), 13.4 (v.s.), 14.32 (s), and 15.1 (m) μ.

The UV spectrum showed $\lambda_{max.}^{MeOH}$ at 250 and 356 nm. (1.5×10^5 and 1.2×10^4, respectively).

Six recrystallizations from chloroform–methanol (1:3) afforded the analytical sample as yellow needles, m.p. 153–155°.

Anal.—Calc. for $C_{14}H_{11}NO$: C, 80.36; H, 5.30; N, 6.69. Found[10a]: C, 79.73; H, 4.93; N, 6.55.

This compound failed to analyze correctly with respect to carbon. Three analytical samples were sent, of which the best results are reported.

Reduction of 9-Hydroxymethylacridine (VII*b*)[9] with Lithium Aluminum Hydride ($LiAlH_4$)—A solution of 0.070 g. (0.00033 mole) of VII*b* in 10 ml. of dry ether was added slowly to a stirred mixture of 0.5 g. of $LiAlH_4$ and 15 ml. of dry ether. The mixture was stirred under nitrogen and at room temperature for 24 hr. At the end of this time, the excess $LiAlH_4$ was decomposed by add watering with cooling (0–5°). The mixture was filtered, and the aqueous layer was extracted with chloroform. The combined extracts were dried over anhydrous K_2CO_3 and distilled under reduced pressure at 35°. The residue was recrystallized once from benzene–hexane to afford 0.054 g. (80% yield) of VII*a*, m.p. 134–136°. One more recrystallization from benzene–hexane afforded white needles, m.p. 135–136°, which were identified as being VII*a* by mixed melting point (135–136°) and IR and UV spectra comparison with a sample of VII*a* prepared according to the procedure of Bergmann and Rabinovitz (8).

REFERENCES

(1) G. A. Digenis, *J. Pharm. Sci.*, **58,** 39(1969).

(2) H. W. Whitlock, Jr., and G. A. Digenis, *Tetrahedron Lett.*, **24,** 1521(1964).

(3) W. Rzeszotarski and Z. Ledochowski, *Rocz. Chem.*, **37,** 1631(1963); through *Chem. Abstr.*, **60,** 14472*c*(1964).

(4) N. Kornblum and D. C. Iffland, *J. Amer. Chem. Soc.*, **71,** 2137(1949).

(5) L. M. Jackman, in "Advances in Organic Chemistry: Methods and Results," vol. II, R. A. Raphael, E. C. Taylor, and H. Wynberg, Eds., Interscience, New York, N. Y., 1960, p. 333.

(6) L. F. Fieser and M. Fieser, "Advanced Organic Chemistry," Reinhold, New York, N. Y., 1961, p. 859.

(7) D. D. Van Slyke, R. T. Dillon, D. A. MacFadyen, and P. Hamilton, *J. Biol. Chem.*, **141,** 631(1941).

(8) E. D. Bergmann and M. Rabinovitz, *J. Org. Chem.*, **25,** 827 (1960).

(9) M. A. Miyamoto, H. B. Kostenbauder, D. Walstad, and G. A. Digenis, *FEBS Lett.*, **7,** 101(1970).

(10) G. A. Digenis, D. L. Walstad, M. Ishaque, and M. I. H. Aleem, *Biochem. Pharmacol.*, **20,** 919(1971).

(11) D. O'Donovan and E. Leete, *J. Amer. Chem. Soc.*, **85,** 461 (1963).

(12) J. W. Daly and B. Witkop, *Angew. Chem., Int. Ed.*, **2,** 425 (1963).

(13) K. Lehmstedt and F. Dostal, *Chem. Ber.*, **72,** 804(1939).

(14) R. E. Bowman and H. H. Stroud, *J. Chem. Soc.*, **1950,** 1342.

(15) F. E. Gould, G. S. Johnson, and A. F. Ferris, *J. Org. Chem.*, **25,** 1658(1960).

(16) O. Follins, *J. Biol. Chem.*, **51,** 389(1922).

ACKNOWLEDGMENTS AND ADDRESSES

Received February 24, 1970, from the **Department of Pharmaceutical Chemistry, University of Kentucky, Lexington, KY 40506* and the *†Department of Chemistry, University of Wisconsin, Madison, WI 53715*

Accepted for publication September 23, 1971.

Presented to the Medicinal Chemistry Section, APhA Academy of Pharmaceutical Sciences, Montreal meeting, May 1969.

The assistance of Miss Diana Walstad in the preparation of this manuscript is gratefully acknowledged.

▲ To whom inquiries should be directed.