Synthesis of the *p*-Nitrophenyl Ester of Acridine-2-acetic Acid

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The title compound, **8**, which is expected to serve as a fluorescent substrate for α-chymotrypsin, was prepared by esterification of acridine-2-acetic acid, **4**, whose synthesis was achieved by two routes. One began with 4-aminophenylacetic acid and proceeded through the acridanone-2-acetic acid, **3**, in 49% yield overall. The other began with 4-aminoacetophenone and proceeded through 2-acetylacridanone, **6**, in 3% yield overall.

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The benzophenone-4-acetyl group, when bound covalently to α-chymotrypsin, was found to be a useful phosphorescent probe for the active site of the enzyme (1). The acridine-2-acetyl group, which is isosteric and nearly isoelectronic with the benzophenone-4-acetyl group, should act as a fluorescent probe for α-chymotrypsin. The synthesis of a compound containing the acridine-2-acetyl group, the *p*-nitrophenyl ester **8**, which should serve as the actual enzyme substrate, is reported here (2).

In the first approach, the known (3) diphenylamine-acetic acid derivative, **1**, was prepared from 4-amino-phenylacetic acid and 2-chlorobenzoic acid by the method used by Pfister (4) *et al.* for the lower homolog, the diphenylaminecarboxylic acid. Pfister used *N,N*-dimethylformamide rather than isoamyl alcohol as the solvent, thus eliminating the need for a tedious steam distillation and extraction; and he used the free benzoic acid rather than its potassium salt, a convenience. With these methods, we obtained **1** in 69% yield (recrystallized) *vs.* 65% (crude) reported (3). Cyclization of **1** with phosphorus oxychloride, a general method (5), did not give the 9-chloroacridine, **2**, as a clean product in this case. Therefore, dehydration of **1** to form the 9-acridanone, **3**, was selected as the first step of an alternate route. While sulfuric acid has been reported by Pfister for the dehydration of the lower homolog in unspecified yield, and thionyl chloride was used by Graboyes (6) *et al.*, for dehydration of several diphenylamines, cyclization of **1** proved especially sensitive to these reagents, as shown in Table I. Polyphosphoric acid

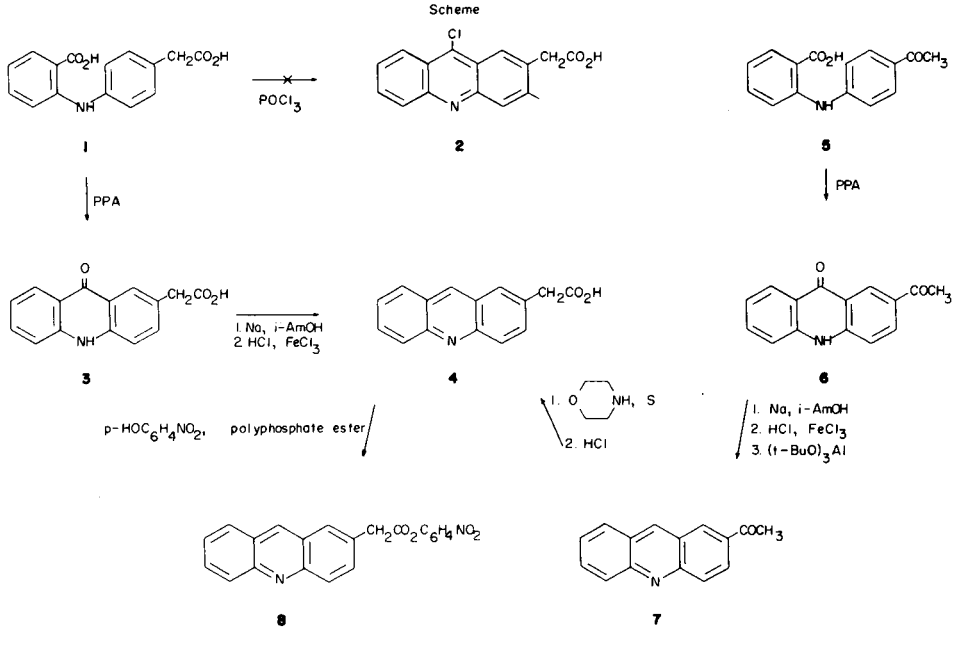
Table I

Effect of Dehydrating Agents on the Yield of 9-Acridanone **3**

Dehydrating Agent	Reaction Time	Reaction Temperature	Yield % (a)
Sulfuric acid	24 hours	20°	0
Sulfuric acid	24 hours	100	28 (b)
Sulfuric acid	3 hours	80-90	48
Thionyl chloride	3	80	0 (c)
PPA	3	80-90	80
PPA	1.5	80-90	96

(a) Monitored by means of the ir peak of acridanone at 1645 cm^{-1}. (b) With charring. (c) Intractable mixture.

Scheme



was finally adopted. Reductions of 9-acridanones by the Wolff-Kishner method and by the sodium in amyl alcohol method were reported without yields for the lower homolog by Pfister. In our hands the Wolff-Kishner procedure gave 40% yield and dissolving sodium gave 79% yield of acridine-2-acetic acid, **4**.

The alternate route *via* acetyldiphenylamine **5** was attempted partly because other 2-acylacridines besides **7** were needed. Condensation of 2-bromobenzoic acid and 4-aminoacetophenone in *N,N*-dimethylformamide gave **5** in 76% yield (recrystallized) *vs.* the reported (7) 57% (crude). Polyphosphoric acid cyclization of **5** furnished the new 2-acetyl-9-acridanone, **6**, in 92% yield. A Wolff-Kishner reduction on the dicarbonyl compound **6** would have been expected to reduce both carbonyl groups with removal of oxygen, thus sodium in amyl alcohol was used; but this gave a mixture of reduced products containing 2-(1-hydroxyethyl)-9-acridanol. Without purification, the mixture was selectively oxidized to 2-(1-hydroxyethyl)-acridine with iron(III) chloride, and then to 2-acetylacridine, **7**, in 22% yield by means of an Oppenauer oxidation of the methylcarbinol function. A Willgerodt reaction with **7** gave the desired thiomorpholide, whose attempted alkaline hydrolysis failed, but whose acid hydrolysis gave **4** in 20% yield from **7**.

The acridine-2-acetic acid, **4**, showed fluorescence emission maxima of equal intensity at 423 and 443 nm at a concentration of $10^{-4}M$ in methanol. Esterification of **4** with *p*-nitrophenol was accomplished with "polyphosphate ester" using the method of Campbell and Gioannini (8) to give ester **8** in 88% yield. Results of its use as a fluorescent probe will be published elsewhere.

EXPERIMENTAL

Capillary melting points were determined in a Thomas-Hoover Unimelt and need no correction. The pmr spectra were obtained with a Perkin-Elmer R12 using TMS as an internal standard. The ir spectra were obtained with a Perkin-Elmer 700 grating instrument, the samples in Nujol mulls or potassium bromide discs; only bands relevant to the confirmation of structure and other strong bands are reported. A Farrand Mk. 2 with 1 and 5 nm slits was used for fluorescence spectra both excitation and emission being corrected. Elemental analyses were carried out by Microanalysis, Inc., Wilmington, Delaware.

N-(4-Carboxymethylphenyl)anthranilic Acid (**1**).

A mixture of 75 ml of *N,N*-dimethylformamide (Baker 9221), 4-aminophenylacetic acid (5.5 g, 0.036 mole, Aldrich 7,135-2), 2-chlorobenzoic acid (5.2 g, 0.036 mole, Aldrich 13,557-7), anhydrous potassium carbonate (15 g, 0.108 mole, Baker 3012), copper powder (0.4 g, Baker 1728), and a trace of copper(I) chloride were heated with magnetic stirring at 140-145° for 6 hours under a stream of dry nitrogen gas, cooled to 70°, and quenched in 200 ml of 1:1 ice:water. The solution was decolorized with 1.5 g of activated carbon (Norit A) at 100° for 5 minutes, filtered, and neutralized with 6*M* hydrochloric acid in an ice bath. The crude crystalline product was recrystallized from 20 ml/g of 95% ethanol to give 6.9 g (69%), mp 207-209° dec (lit (3) 210°); pmr (8% in DMSO-d_6): δ 3.44 (2H, s, CH_2), 5.6-5.9 (1H, broad s, NH), 6.72-8.14 (8H, m, arom. H), 9.45 (1H, s, RCO_2H), 11.82 (1H, s, $ArCO_2H$); ir: ν min 1708, 1675, 1610, 1345, 920 cm^{-1}.

Anal. Calcd. for $C_{15}H_{13}NO_4$: C, 63.69; H, 4.83. Found: C, 63.78; H, 4.84.

Acridanone-2-acetic Acid (**3**).

Polyphosphoric acid (20 ml, Eastman 15392) was heated to 80-90° with magnetic stirring in a hot water bath while *N*-(4-carboxymethylphenyl)-anthranilic acid (**1**, 2.0 g) was added during 15 minutes. After an additional 1.5 hours at 90°, the reaction mixture was cooled to 40° and poured into 100 ml of ice-water (exothermic), and the resulting suspension was boiled for 5 minutes, cooled to 20°, and filtered to yield 1.8 g (96%) of crude product. Recrystallization from acetic acid, 15 ml/g gave 1.65 g (89%) of yellow-ochre flakes, mp 299-301° dec; pmr (10% in DMSO-d_6): δ 3.75 (2H, s, CH_2), 6.10 (1H, broad s, NH), 6.90-7.95 (7H, m, arom. H), 11.81 (1H, s, CO_2H); ir: ν min 1708, 1645, 1610, 1345, 1280, 760 cm^{-1}.

Anal. Calcd. for $C_{15}H_{11}NO_3$: C, 71.12; H, 4.38; Neut. Equiv. 253. Found: C, 70.86; H, 4.41; Neut. Equiv. 256.

Acridine-2-acetic Acid (**4**).

Sodium metal (1.5 g, 0.065 mole, Baker 9412) was added in small pieces during 15 minutes to a mixture of 9-acridanone-2-acetic acid (**3**, 3.0 g, 0.012 mole) in 35 ml of isoamyl alcohol with magnetic stirring. Heating to 112° caused a vigorous reaction with frothing. The mixture was further heated under reflux for 1 hour, resulting in complete solution of the sodium. Solvent was removed by steam distillation, and the aqueous residue was brought to *p*H 7 with 6*N* hydrochloric acid. This was followed by addition of 20 ml of saturated iron(III) chloride, boiling for 5 minutes, and further acidification with 6*M* hydrochloric acid to precipitate the crude product, which was recrystallized from 45 ml/g of 95% ethanol to give golden-yellow crystals, 2.2 g (79%), mp 270-271°; pmr (9% in DMSO-d_6): δ 3.81 (2H, s, CH_2), 7.0-7.82 (8H,m, arom. H), 11.65 (1H, s, CO_2H); ir: ν min 1704, 1612, 1510, 1470, 1145, 794 cm^{-1}; fluorescence ($1.1 \times 10^{-4}M$ in methanol): λ max (exc) 278 (0.45), 299 (0.29), 312 (0.24), 383 sh (0.66), 400 nm (0.72 relative intensity); λ max (em) 423 (0.55), 433 (0.55), 470 sh nm (0.28 relative intensity).

Anal. Calcd. for $C_{15}H_{11}NO_2$: C, 75.95; H, 4.64; Neut. Equiv., 237. Found: C, 75.47; H, 4.55; Neut. Equiv., 241.

N-(4-Acetylphenyl)anthranilic Acid (**5**).

The method used above for **1** was applied to 2-bromobenzoic acid and 4-aminoacetophenone to yield 76% of **5**, mp 172-173° (lit (7) 174°).

Anal. Calcd. for $C_{15}H_{13}NO_3$: C, 70.59; H, 5.09. Found: C, 70.86; H, 5.19.

2-Acetyl-9-acridanone (**6**).

The method used above for **3** was applied to **5** to give 92% of **6**, mp 306-307°; pmr (DMSO-d_6): δ 2.55 (3H, s, CH_3), 5.92-6.30 (1H, broad s, NH), 6.94-8.12 (7H, m, arom. H); ir: ν min 3310, 1646, 1635, 1630, 1345, 1160 cm^{-1}.

Anal. Calcd. for $C_{15}H_{11}NO_2$: C, 75.95; H, 4.64. Found: C, 75.47; H, 4.65.

2-Acetylacridine (**7**).

The method used above to reduce **4** was applied to 2-acetyl-9-acridanone (**6**, 1.2 g, 0.0050 mole). The aqueous residue, after treatment with iron(III) chloride, deposited a brown semi-solid that was triturated with ether. The resulting solid was shown by tlc on alumina developed with methanol:chloroform:benzene 3:3:1 to contain at least three components, and these were not easily resolved by column chromatography. The dried brown solid was stirred with 20 ml of dry acetone (9), then 20 ml of sodium-dried toluene was added followed by a solution of 1.9 g of aluminum *t*-butoxide (9) in 10 ml of dry toluene. The reaction mixture was boiled under reflux for 12 hours, cooled, decomposed with 25 ml of 3*M* hydrochloric acid, and allowed to separate into layers. The dark toluene layer was dried over calcium chloride and evaporated. The residue was chromatographed on a 25 cm column of neutral alumina by eluting with methanol:chloroform:benzene 2:3:1. The first fraction, deep yellow, was a mixture. The second fraction, bright yellow, was crude **7**, 0.25 g (22%), mp 151.5-153.5°; pmr (9% in DMSO-d_6): δ 2.51 (3H, s, CH_3), 6.95-8.05 (8H, m, arom. H); ir: ν min 1680, 1605, 1471, 1145, 895, 830 cm^{-1}.

The analytical sample was recrystallized from ethyl acetate, mp 152.5-153.5°.

Anal. Calcd. for $C_{15}H_{11}NO$: C, 81.42; H, 4.98. Found: C, 81.72; H,5 .19.

Acridine-2-acetic Acid, *p*-Nitrophenyl Ester (**8**).

The procedure of Campbell and Gioannini (8) was carried out upon acid **4**, the crude semi-solid product needing trituration with ether before recrystallization from 20 ml/g of 95% ethanol to give 88% of **8**, mp 201-202°; pmr (8% in deuteriochloroform): 3.86 (2H, s, CH_2), 7.10-8.15 (12H, m, arom. H); ir: ν min 1760, 1545, 1250, 1140 cm^{-1}.

Anal. Calcd. for $C_{21}H_{14}N_2O_4$: C, 70.38; H, 3.91. Found: C, 69.97; H, 4.12.

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