POPOP in a liter of toluene. Controls with different known concentrations of the labeled drug were run concurrently with the experiment to serve as a check on the overall technique. All samples were counted for sufficient time to yield <2.5% error.

Extracts to be examined by glc were concentrated under reduced pressure and the residues were dissolved in acetone. The analysis was performed on a 6 ft × 0.25 in. od column packed with 3% OV-17 on chromosorb W (80–100 mesh) at 180–220° using N_2 as carrier gas.

Tlc was performed on basic alumina sheets (Eastman chromagram 6062) in one and two dimensions using EtOAc or Et_2O as solvent. The samples were mixed with authentic samples of nonlabeled carriers and applied to the plates. The spots were visualized with iodine reagent (0.5% I_2 in $CHCl_3$). The plates were divided into strips and the radioactivity on each strip was determined by scraping the adsorbent into a counting vial, adding toluene scintillation cocktail, and counting directly.

Brain and Plasma Levels. Adult male Swiss-Webster mice (25–30 g) were decapitated and exsanguinated at various times after sc administration of various doses of the labeled compounds. Blood was collected in beakers containing heparin sodium powder, transferred to tubes, and immediately centrifuged. Brains were removed immediately after decapitation, rinsed quickly with 3–5 ml of normal saline, blotted dry, and weighed. The weighed brains were then transferred to 15-ml glass homogenizer vessels, mixed with sufficient 0.01 *N* HCl to make a total volume of 5 ml, and homogenized. An aliquot of brain homogenate or plasma was adjusted to pH > 12, mixed with twice its volume of benzene, shaken, and centrifuged. An aliquot of the C_6H_6 phase was mixed with 0.1 *N* HCl, shaken, and centrifuged. The C_6H_6 phase, after washing with 0.1 *N* HCl, was used for analysis of the N-demethylated metabolite. The aqueous acid phase was washed with C_6H_6, basified, and extracted with C_6H_6. The C_6H_6 phase was used for the analysis of the unchanged drug. Controls with different known concentrations of the labeled drug were run concurrently to serve as a check on the overall technique, and a straight line was obtained when absolute counts per min were plotted against concentration. Percentage recoveries of labeled drugs were 90–95% from brains and plasma. The identity of the radioactive material in the benzene extracts was determined by tlc on basic alumina (EtOAc or Et_2O as solvents).

Plasma Protein Binding Studies. The per cent bound to mouse plasma protein was determined by equilibrium dialysis. Blood collected from 8–10 Swiss-Webster male mice was centrifuged at 9000*g* for 15 min. The plasma (0.4 ml) was placed inside a 0.5 × 3 cm tube of hydrated cellulose dialysis membrane which was stoppered at both ends with glass plugs. The filled tube then was placed in a test tube containing a sufficient vol of [^{3}H]prodine isomer in isotonic pH 7.4 phosphate buffer to make the internal and external liquid levels equal. The range of concentrations (0.25, 0.5, 1.0 μg/ml) of each [^{3}H]proidine isomer was in the vicinity of the peak plasma levels found *in vivo* at their ED_{50} doses. The dialysis assembly was immersed in a water bath (30°) which was agitated by an eccentric rotor for 15 hr (twice the time required for equilibrium). Dialysis at each concentration was performed in duplicate or triplicate. Analyses were carried out on 250-μl aliquots of the buffer and plasma phases by liquid scintillation spectrometry. Verification (tlc) of the radiolabeled material after dialysis indicated the absence of ester hydrolysis products. The per cent of protein-bound [^{3}H]prodines was calcd from the difference between the concentrations on the two sides of the dialysis membrane.

References

(1) P. S. Portoghese and T. N. Riley, *J. Med. Chem.*, 14, 561 (1971) (paper 10).
(2) (a) *Pharmacologist*, 12, 175 (1970); (b) presented in part at the 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1970, Abstract MEDI 46.
(3) P. S. Portoghese, *J. Pharm. Sci.*, 55, 865 (1966).
(4) P. S. Portoghese, *Annu. Rev. Pharmacol.*, 10, 51 (1970).
(5) C. Sung and E. L. Way, *J. Pharmacol. Exp. Ther.*, 109, 244 (1953).
(6) P. A. Shore, J. Axelrod, C. A. M. Hogben, and B. Brodie, *ibid.*, 113, 192 (1955).
(7) N. A. Ingoglia and V. P. Dole, *ibid.*, 175, 84 (1970).
(8) B. A. Berkowitz and E. L. Way, *ibid.*, 177, 500 (1971).
(9) P. S. Portoghese, A. A. Mikhail, and H. J. Kupferberg, *J. Med. Chem.*, 11, 219 (1968).
(10) P. S. Portoghese and D. L. Larson, *J. Pharm. Sci.*, 57, 711 (1968).
(11) D. L. Larson, Ph.D. Thesis, University of Minnesota, 1969.
(12) A. Ziering and J. Lee, *J. Org. Chem.*, 12, 911 (1947).
(13) P. J. Janssen and N. B. Eddy, *J. Med. Pharm. Chem.*, 2, 31 (1960).
(14) (a) J. F. Cavalla, R. A. Selway, J. Wax, L. Scotti, and C. V. Winder, *ibid.*, 5, 441 (1962); (b) R. E. McMahon, A. S. Ridolfo, H. W. Culp, R. S. Wolen, and F. J. Marshall, *Pharmacologist*, 12, 301 (1970).
(15) B. G. Boggiano, V. Petrow, O. Stephenson, and A. M. Wild, *J. Chem. Soc.*, 1143 (1959).
(16) A. F. Casy, M. A. Loris, and P. Pocha, *ibid.*, 942 (1967).
(17) J. Cochin and J. Axelrod, *J. Pharmacol. Exp. Ther.*, 125, 105 (1959).
(18) B. N. Wilkinson, *Biochem. J.*, 80, 324 (1961).
(19) J. Axelrod, *J. Pharmacol. Exp. Ther.*, 117, 322 (1956).
(20) A. Goldstein, "Biostatistics," Macmillan Co., New York, N. Y., 1964.
(21) E. Kutter, A. Herz, H.-J. Jeschemacher, and R. Hess, *J. Med. Chem.*, 13, 801 (1970).

Synthesis of α-[*p*-(Fluoren-9-ylidenemethyl)phenyl]-2-piperidineethanol, an Inhibitor of Platelet Aggregation

George P. Claxton, J. Martin Grisar,* Edward M. Roberts, and Robert W. Fleming

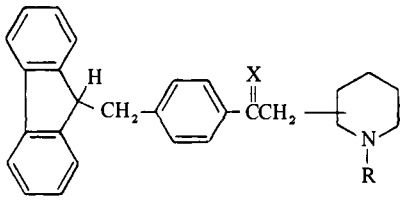
Organic Chemistry Department, Merrell-National Laboratories, Division of Richardson-Merrell Inc., Cincinnati, Ohio 45215.
Received November 18, 1971

Inhibition of blood platelet aggregation has been demonstrated among a number of pyridine- and piperidineethanols of benzyl- and benzylidenefluorene **1-15**. In preparation for clinical evaluation of some of these compounds, one of the diastereoisomers of α-[*p*-(fluoren-9-ylidenemethyl)phenyl]-2-piperidineethanol (**5**) was selected for further evaluation as an inhibitor of aggregation of human blood platelets. An improved synthesis of **5** is reported in which a novel Mannich-type reaction of 2,3,4,5-tetrahydropyridine trimer (**25**) and the complex **24** of 4′-(fluoren-9-ylidenemethyl)acetophenone (**19**) with magnesium methyl carbonate in dimethylformamide to give **6** is used.

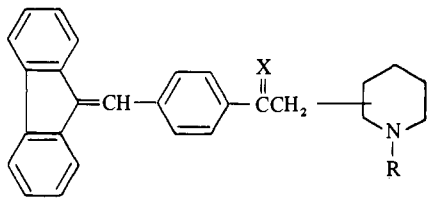
As an extension of our work on pyridine- and piperidineethanols and Me ketones in the triphenylethane and triphenylethylene series,[1] we prepared **1** through **15** containing the benzyl- and benzylidenefluorene moiety.[2] Relatively early during the biological evaluation, **5** was found to prolong whole-blood clotting time in rats. It was subsequently found that at a concentration of 30 μg/ml **5** inhibits *in vitro* platelet aggregation (human citrated blood) induced by adenosine diphosphate (ADP), collagen, or thrombin. Compound **5** also inhibited clot retraction.[3,4] Still later, **5** was shown to antagonize ADP-induced thrombocytopenia in guinea pigs *in vivo* at 30 mg/kg po.[5] In this system, the two diastereoiso-

mers of **5** differed and the higher melting isomer was shown to be superior.† Of the congeners, **14** also showed anticoagulant activity as determined by rat whole-blood clotting time; it did not, however, affect human platelet function *in vitro*. All of the piperidine congeners **1–8** inhibited *in vitro* ADP-induced platelet aggregation at 30 to 100 μg/ml. However, a significant difference was seen in their tendency to release, at higher concentrations, platelet factor 3 (PF3), which enhances coagulation.[6] On this basis, **5** was selected for further evaluation, since it showed less than 0.2% of PF3 release at 300 μg/ml.[3,4]

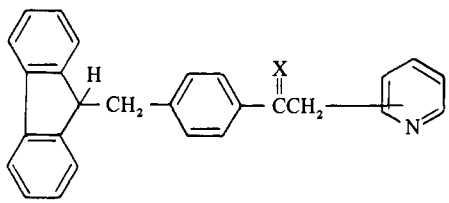
At this point it became important to find a good synthesis for **5**. The reactions of 2- or 4-picolyllithium or Na[7] with



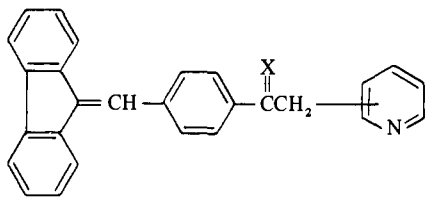
1, X = OH, H; 2-piperidyl; R = H
2, X = OH, H; 4-piperidyl; R = H
3, X = O; 4-piperidyl; R = H
4, X = OH, H; 4-piperidyl; R = CH_3



5, X = OH, H; 2-piperidyl; R = H
6, X = O; 2-piperidyl; R = H
7, X = OH, H; 3-piperidyl; R = CH_3
8, X = O; 3-piperidyl; R = CH_3



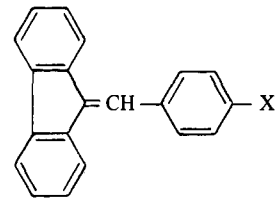
9, 2-pyridyl; X = O
10, 2-pyridyl; X = OH, H
11, 4-pyridyl; X = O
12, 4-pyridyl; X = OH, H



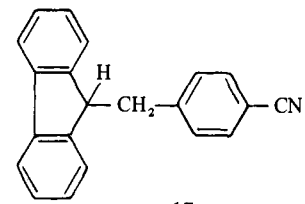
13, 2-pyridyl; X = O
14, 2-pyridyl; X = OH, H
15, 2-pyridyl; X = OH, CH_3

α-fluoren-9-yl-*p*-tolunitrile (**17**) had given reasonably good yields of **9** and **11**. The reaction with the unsaturated nitrile **16**, however, gave poor results; after much experimentation, the maximum yield of **13** was 35%. The reaction of aldehyde **18**, which was obtained from **16** in 35–50% yield, with 2-picolyllithium gave only 28% of **14**. The low yields are partly explained by the isolation of the bis adducts **20–23**. Their structure is assigned on the basis of an analogy re-

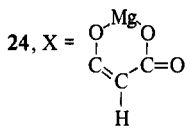
†This isomer of 5 is known under the code name RMI 10,393.



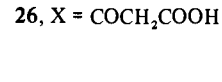
16, X = CN
18, X = CHO
19, X = $COCH_3$



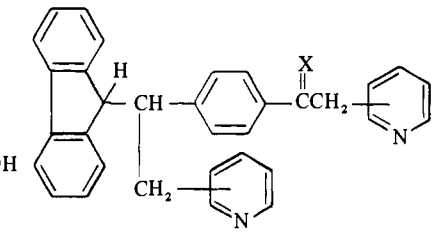
17



24, X =



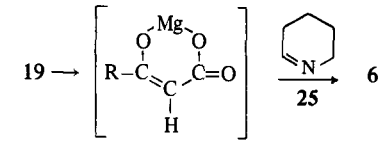
26, X = $COCH_2COOH$



20, 2-pyridyl; X = O
21, 2-pyridyl; X = OH, H
22, 4-pyridyl; X = O
23, 4-pyridyl; X = OH, H

ported by Ziegler and Schäfer, who found that PhLi adds to benzylidenefluorene to give benzhydrylfluorene,[8] and on the basis of analytical and spectral data (see Experimental Section). The fact that one of the reduction products **21** and **23** could be separated into diastereoisomers further confirms the point of addition to the double bond. These bis adducts were isolated in yields as high as 26%, depending on reaction conditions. Finally, we found that in spite of much effort, the reduction of the pyridine ring of **14** could not be controlled to avoid partial reduction also of the double bond, the result being a mixture of **5** and **1**. Since both these compounds occur as pairs of diastereoisomers with similar properties, the separation of the **5** isomers from this mixture proved impractical. At this point, we decided to explore other synthetic routes, in particular, one in which the saturated piperidine moiety is introduced into the molecule.

We succeeded in preparing **5** in excellent yield by a novel route. Reaction of the methyl ketone **19** with magnesium methyl carbonate[9-11] gave a complex of presumed structure **24** in DMF to which the trimer of 2,3,4,5-tetrahydropyridine (**25**)[12-14,‡] was added. In an atm of CO_2 at room temp,



24

the Mannich product **6** formed within 10–20 hr and was isolated as the HCl salt in 70–90% yield. The ketone **6** was reduced to **5** with $NaBH_4$. One of the diastereoisomers crystallized preferentially as neutral fumarate salt and was readily purified while the other diastereoisomer was obtained and purified as free base from the mother liquors. Both isomers were converted to glycolate salts since these were found to be more readily sol in H_2O.

The work of Schöpf and coworkers on the reaction of β-keto acids with 2,3,4,5-tetrahydropyridine (**25**) in aq sol at

‡2,3,4,5-Tetrahydropyridine trimerizes in two stereoisomeric forms designated as α- and β-tripiperideine. We generally used α-tripiperideine but found that the β isomer undergoes condensation equally well.

pH values below 11 to give 2-piperidylmethyl ketones has contributed significantly to the understanding of chemical reactions under "physiological" conditions as well as to that of pathways of biogenesis of a large number of plant alkaloids containing piperidine moieties.[15,16] However, the reaction has not found wide synthetic application and has only been used with lower alkanoyl- and benzoylacetic acids.[16-19] One limitation of the reaction is the lack of water solubility of β-keto acids with larger substituents, especially when the reaction is carried out at acidic pH;[17] another is the requirement of pH control during the reaction to avoid self-condensation of 2,3,4,5-tetrahydropyridine (**25**).[18-20] Thus, an attempt to prepare **6** from **25** and the β-keto acid **26** in a variety of solvent systems (H_2O, 90% DMF, 95% and 100% MeOH) and at varying pH failed to give more than 6% of product. Direct Mannich condensations of 2,3,4,5-tetrahydropyridine (**25**) with indole[21,22] and pyrrole[23] have also been reported. Therefore, an attempt was made to condense **25** with the Me ketone **19** under a variety of reaction conditions, but only very small amounts of **6** were obtained.

These synthetic studies indicate that the use of the chelate **24** formed by reaction of the aryl methyl ketone **19** and magnesium methyl carbonate overcomes the practical limitations of the Schöpf condensation imposed by lack of solubility and the need for pH control. Chelation may also provide an additional driving force for the reaction as suggested by Stiles for alkylation reactions.[11] The generality of the reaction is being explored.

Experimental Section§

α-Fluoren-9-yl-*p*-tolunitrile (17). A soln of α-fluoren-9-ylidine-*p*-tolunitrile (**16**)[24,25] in 600 ml of DMF was shaken under H_2 with 3.0 g of Pd/C. Concn of filtrate gave 73 g of **17**, mp 170–172°, that was recrystd from C_6H_6, mp 171–173°. *Anal.* ($C_{21}H_{15}N$) C, H, N.

2-[*p*-(Fluoren-9-ylmethyl)phenacyl]pyridine (9). To a soln of PhLi, prepd from 84.4 g of PhBr and 7.5 g of Li in 600 ml of abs Et_2O, was added 50.1 g of α-picoline in 125 ml of Et_2O over 1 hr and refluxed for 1.5 hr. The Et_2O was replaced by 800 ml of C_6H_6. A warm soln of 81.6 g of α-fluoren-9-yl-*p*-tolunitrile (**17**) in 1 l. of dry C_6H_6 was added to the hot, stirred picolyllithium soln in a 10-min period. The reaction mixt was heated at reflux, with stirring, for 1.5 hr and allowed to stand at room temp overnight. About 250 ml of 10% HCl was added and stirred with the reaction mixt for 2 hr. The mixt was then made basic with dil NaOH. The organic layer was sepd, washed thoroughly with H_2O, dried (Na_2SO_4), and concd to about 400 ml; 38.3 g of crude product, mp 133–137°, was obtd. Three recrystns from C_6H_6 gave 11.8 g (11%) of **9**, mp 156–157.5°. *Anal.* ($C_{27}H_{21}NO$) C, H, N.

α-(Fluoren-9-yl-*p*-tolyl)-2-pyridineethanol (10). A soln of 14.2 g of **9** in 800 ml of 3:1 EtOH–THF was treated with 7.0 g of $NaBH_4$, added in portions with stirring. After several hours, the mixt was poured into 2 l. of ice water and the product was collected by filtration. Two recrystns from C_6H_6 gave 7.7 g of **10**, mp 140–141°. *Anal.* ($C_{27}H_{23}NO$) C, H, N.

4-[*p*-(Fluoren-9-ylmethyl)phenacyl]pyridine (11). 4-Picolylsodium was prepd in 200 ml of 4-picoline with 10 g of $NaNH_2$ according to the method of Wright, *et al.*[26] A soln of 42.2 g of **17** in 600 ml of 4-picoline was added rapidly to the cooled picolylsodium soln. The reaction mixt was allowed to warm to room temp and then stirred for 2.5 hr. It was poured into H_2O, the resulting oil was sepd, washed again with H_2O, and heated with 600 ml of 1 *N* HCl on a steam bath for 0.5 hr. The soln was neutralized with 2 *N* NaOH, and the product was extd into $CHCl_3$, washed thoroughly with H_2O, and dried. The resulting oil (64.6 g) crystd from C_6H_6 and gave 43.7 g, mp 101–110°. Two recrystns from C_6H_6 gave 18.3 g (33%) of **11**, mp 125–126°. *Anal.* ($C_{27}H_{21}NO$) C, H, N.

α-(Fluoren-9-yl-*p*-tolyl)-4-pyridineethanol (12). $NaBH_4$ reduction of **11** by the procedure described for the 2 isomer (**10**) gave **12** in 92% yield, mp 175–177°. *Anal.* ($C_{27}H_{23}NO$) C, H, N.

4-[*p*-(Fluoren-9-ylmethyl)phenacyl]piperidine (3). Compd **11** (15.0 g) was hydrogenated in DMF over PtO_2 (1.0 g) in the presence of 2 equiv of alcoholic HCl. The reduction stopped at the ketone stage in spite of prolonged shaking and slight warming. The product was isolated as free base (14.5 g) and reconverted to HCl salt. Two recrystns from MeOH–EtOAc gave 8.0 g (48%) of **3**, mp 242–244°, ν 1675 cm^{-1}. *Anal.* ($C_{27}H_{27}NO \cdot HCl$) C, H, Cl.

α-[α-(Fluoren-9-yl)-*p*-tolyl]-4-piperidineethanol (2). $NaBH_4$ reduction of **3** (5.0 g) in abs EtOH gave **2** (4.5 g) that was converted to HCl salt and recrystd from MeOH–EtOAc (1.8 g), mp 203–204°. *Anal.* ($C_{27}H_{29}NO \cdot HCl$) C, H, Cl.

α-(Fluoren-9-yl-*p*-tolyl)-1-methyl-4-piperidineethanol (4). A mixt of 9.5 g of **11** and 5 ml of MeI in 60 ml of MeOH in a sealed pressure bottle was heated on a steam bath for 2.5 hr. The mixt was poured into Et_2O and the resulting ppt was recrystd twice from EtOH to give 8.7 g of 4-[*p*-fluoren-9-ylmethyl)phenacyl]-1-methylpyridinium iodide, mp 210–212°. *Anal.* ($C_{28}H_{24}INO$) C, H, N. This material (6.1 g) was hydrogenated in a Parr shaker in DMF over 1.0 g of PtO_2 and the crude product was further reduced with $NaBH_4$ in abs EtOH to give, after 3 recrystns from Et_2O, 2.8 g (61%) of **4**, mp 161–162°. *Anal.* ($C_{28}H_{31}NO$) C, H, N.

α-(α-Fluoren-9-ylidene-*p*-tolyl)-1-methyl-3-piperidineethanol (7). A Grignard reagent of 3-chloromethyl-1-methylpiperidine (31.4 g) was prepd in THF and the solvent replaced by toluene. A soln of 30.2 g of α-fluoren-9-ylidene-*p*-tolualdehyde (**18**) in 500 ml of toluene was added and the mixt was refluxed for 4.5 hr. The product obtd after decompn with 3 *N* NH_4Cl was chromatogd to remove impurities and converted to the dihydrogen citrate salt, 17.4 g (28% yield), mp 117–120°. *Anal.* ($C_{28}H_{29}NO \cdot C_6H_8O_7$) C, H, N. The product is believed to be a mixt of diastereoisomers.

3-[*p*-(Fluoren-9-ylidenemethyl)phenacyl]-*N*-methylpiperidine (8). Grignard reaction of α-fluoren-9-ylidene-*p*-tolunitrile (**16**) and 3-magnesiomethyl-1-methylpiperidine chloride in toluene gave **8**, mp 83–90°. *Anal.* ($C_{28}H_{27}NO$) C, H, N.

α-Fluoren-9-ylidene-*p*-tolualdehyde (18). Anhyd $SnCl_2$ was freshly prepd by stirring 226 g of $SnCl_2 \cdot 2H_2O$ with 208 g of Ac_2O for 2 hr with cooling in an ice bath. It was collected, washed with anhyd Et_2O, and placed in a reaction vessel under 400 ml of anhyd Et_2O and 500 ml of $CHCl_3$. Anhyd HCl was bubbled into this suspension for 4.5 hr to saturate the system. Then a soln of 100 g of α-fluoren-9-ylidene-*p*-tolunitrile (**16**) in 600 ml of $CHCl_3$ was added rapidly and slow introduction of anhyd HCl was contd for 2–3 hr. The reaction mixt solidified and the bright yellow ppt was collected and washed with anhyd Et_2O and with $CHCl_3$. Thorough washing at this point is advantageous since it removed unreacted nitrile from the imido chloride-Sn complex. Decompn of the complex by heating on a steam bath for 45 min with 1.5 l. of 1% HCl gave 51.8 g of **18**, contaminated with less than 10% of starting nitrile **16**. Further purification was effected by chromatography on a silica gel column. The starting nitrile was eluted with 3:2 pentane–PhH and the aldehyde **18** with PhH to give 37.2 g, mp 118–121°. *Anal.* ($C_{21}H_{16}O$) C, H.

2-[*p*-(Fluoren-9-ylidenylmethyl)phenacyl]pyridine (13).# An ethereal 2-picolyllithium soln, prepd from 50.1 g of α-picoline and PhLi as described above, was added over 30 min to a soln of 100 g of α-fluoren-9-ylidene-*p*-tolunitrile (**16**) in 2 l. of dry toluene. After standing at room temp overnight, the mixt was treated with 1 l. of hot 1 *N* HCl to hydrolize the imine and the product was extd with $CHCl_3$ after neutrn with dil NaOH. Recrystn from butanone gave 46.5 g (35%) of crude **13**, mp 164–170°. Further recrystn raised the mp to 170–172°, ν 1675, 1630 cm^{-1}, λ_{max} ($CHCl_3$) 259 (ε 96,000); 297 (30,300); 359 mμ (54,200). *Anal.* ($C_{27}H_{19}NO$) C, H, N. From the mother liquors, a small amt (6% yield) of the bis adduct **20** was isolated, mp 197–200°, ν 1680, 1625 cm^{-1}; λ_{max} ($CHCl_3$) 263 (ε 32,600), 303 (9500), 343 mμ (7350). *Anal.* ($C_{53}H_{26}N_2O$) C, H, N. This product was obtd in 26% yield when excess α-picolylsodium in α-picoline at room temp was employed. $NaBH_4$ reduction of this material gave a mixt of diastereoisomers of **21**; these were sepd as free bases by tedious recrystn from MeCN, mp 151–153° (*Anal.* ($C_{33}H_{28}N_2O$) C, H, N) and mp 140–142° (*Anal.* ($C_{33}H_{28}N_2O$) C, H, N). Ir, uv, and nmr spectra were in agreement with the assigned structure.

Reaction of α-Fluoren-9-ylidene-*p*-tolunitrile (16) with 4-Picolylsodium. Reaction of **16** with excess 4-picolylsodium in 4-picoline as solvent at 0° gave a nitrile-free product. Since it failed to cryst, it was reduced with $NaBH_4$. Two products were isolated by chromatography and fractional recrystn that are assigned structure **23** (diastereoisomers), mp 220–222° (*Anal.* ($C_{33}H_{28}N_2O$) C, H, N) and mp 190–192° (*Anal.* ($C_{33}H_{28}N_2O$) C, H, N). The pure compds were obtd

§ Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for these elements or functions were within ±0.4%. Melting points were determined on a Hoover capillary melting point apparatus and are corrected.

We are indebted to Mr. D. L. Wenstrup for this experiment.

in 12% yield but, due to the extensive purification required, the actual yield can be estimated to have been at least twice that amt.

α-(α-Fluoren-9-ylidene-*p*-tolyl)-2-pyridineethanol (14). A. From 2-[*p*-(Fluoren-9-ylidenylmethyl)phenacyl]pyridine (13). $NaBH_4$ reduction of 13 in 3:1 EtOH-DMF gave 14, mp 140–141°, in 55% yield. It was converted to the HCl salt, mp 207–209°. *Anal.* ($C_{27}H_{21}NO \cdot HCl$) C, H, N, Cl.

B. From α-Fluoren-9-ylidene-*p*-tolualdehyde (18). The reaction of α-picolyllithium and the aldehyde 18 (carried out in the manner described for 9) gave 14 in 3 and 28% yield.

4′-Fluoren-9-ylidenylmethylacetophenone (19). A hot soln of 100.0 g of α-fluoren-9-ylidene-*p*-tolunitrile (16) in 2 l. of toluene was added rapidly to CH_3MgI (prepared from 82.0 g of CH_3I in Et_2O) in 1 l. of toluene. After refluxing for 2 hr the mixt was decompd with 3 *N* HCl. The crude product was recrystd from CH_3CN, 87.1 g (82%), mp 123–126°. Further recrystn raised the mp to 124–126°. *Anal.* ($C_{22}H_{16}O$) C, H.

α-[α-(Fluoren-9-ylidene-*p*-tolyl)]-α-methyl-2-pyridineethanol (15). Ethereal α-picolyllithium, prepared from 50.1 g of α-picoline and PhLi, was added to a soln of 127.7 g of 19 in 1.3 l. of PhH. The mixt was refluxed for 30 min and allowed to stand overnight. The product was purified by pptn of the HCl salt from anhyd Et_2O and chromatography of the free base, 47.2 g (28%), mp 139–141°. *Anal.* ($C_{28}H_{23}NO$) C, H, N.

4′-(Fluoren-9-ylidenemethyl)-2-(2-piperidyl)acetophenone·HCl (6). A. From 4′-(Fluoren-9-ylidenemethyl)acetophenone, α-Tripiperideine, and Magnesium Methyl Carbonate. Mg methyl carbonate[10] (approx 0.4 mole in 1 *M* soln in dry DMF) was heated to 120° under a stream of CO_2 and 25.0 g (0.084 mole) of the Me ketone 19 was added. N_2 was substituted for CO_2 and the mixt was stirred at 120° for 4 hr while allowing MeOH, that is formed, to escape. The reaction mixt was then again put under dry CO_2 (this is important) and was allowed to cool to room temp, and 7.9 g (0.096 mole of monomer) of α-tripiperideine[12-14]‡ (mp 58–62°) was added as finely ground powder. The mixt was stirred under CO_2 at room temp for 24 hr (or up to 3 days). It was then poured into approx 1 l. of 3 *N* HCl and the solid that resulted after about 3 hr of vigorous stirring was collected, washed with 2 *N* HCl (2 × 40 ml) and Et_2O (3 × 80 ml), and recrystd from 90% and 60% aq *i*-PrOH, respectively, 25.3 (73%), mp 223.5–224° dec. *Anal.* ($C_{27}H_{25}NO \cdot HCl$) C, H, Cl. The reaction was scaled up to 2 moles without difficulty and equal or better yields were obtd.

B. From 4′-(Fluoren-9-ylidenemethyl)acetophenone and α-Tripiperideine. Reaction of equimolar amts of 19 and α-tripiperideine in refluxing *i*-PrOH (3 hr) contg 1.5 equiv of HCl gave 6 in 6% yield.

C. From *p*-(Fluoren-9-ylidenemethyl)phenacylacetic Acid (26) and α-Tripiperideine. The keto acid 26 was obtd by hydrolysis of the complex 24 of 19 with Mg methyl carbonate, mp 115–116.5° dec, ν 1725, 1680 cm^{-1}. The compd decompd on drying in high vacuum and a microanalysis could not be obtd. Reaction with α-tripiperideine in sufficient 1 *N* NaOH to maintain pH of 11 at room temp for 24 hr gave a 7% yield of 6. Using mixts of MeOH and H_2O, or anhyd MeOH, or DMF, gave even less or no product.

D. From α-[*p*-(Fluoren-9-ylidenemethyl)phenyl]-2-piperidineethanol (5). Ice-cold Cornforth reagent,[27,28] prepd from 1.3 g of CrO_3, 1.8 ml of H_2O, and 30 ml of pyridine, was added dropwise to 5.0 g of 5 (free base, isomer mp 142–144°) in 30 ml of pyridine and the mixt was stirred at room temp for 72 hr and turned black. It was poured into H_2O made alk with $NaHCO_3$, and the product was extd into Et_2O and was converted to HCl salt in aq *i*-PrOH, 2.1 g (39%), mp 224–225° dec. Repetition did not improve the yield nor did the use of Sarrett reagent or CrO_3 in 90% AcOH.[28]

α-[*p*-(Fluoren-9-ylidenmethyl)phenyl]-2-piperidineethanol (5). To 14.5 g of $NaBH_4$ in 1.5 l. of abs EtOH was added 84.6 g of 6 in 1 hr. The mixt was stirred for 2 hr at room temp and was then poured into 3.5 l. of ice water. The ppt was collected and treated with 1.4 l. of 10% AcOH to liberate any B complex that may have formed. After neutrn with NaOH, the product was extd into $CHCl_3$, the ext was washed, dried (Na_2SO_4), and the solvent was evapd to give 75 g (97% of theory) of crude semisolid mixt of isomers.

This mixt (0.197 mole) was dissolved in 600 ml of hot EtOH and a soln of 13.3 g (0.114 mole) of fumaric acid in 250 ml of EtOH was added while hot. On cooling, 35.4 g of one isomer was obtd as neutral fumarate salt that was crystd from *i*-PrOH–H_2O to constant mp 240–241° dec. *Anal.* ($C_{27}H_{27}NO \cdot 0.5C_4H_4O_4$) C, H, N. This isomer was obtd in approximately 25% yield from 6, free base, mp 142–144°. *Anal.* ($C_{27}H_{27}NO$) C, H, N. The glycolate salt, mp 126–129° dec (*Anal.* ($C_{27}H_{27}NO \cdot C_2H_4O_3$) C, H, N), was found to be approx 10 times more soluble in H_2O (1:120) than the fumarate salt. The mother liquor of the above isomer was evapd to dryness; the salt was converted to free base and was recrystd from *i*-PrOH to constant mp 151–152.5°. *Anal.* ($C_{27}H_{27}NO$) C, H, N. This isomer† was obtd in approximately 20% yield from 6, neutral fumarate salt, mp 241–243° dec. *Anal.* ($C_{27}H_{27}NO \cdot 0.5C_4H_4O_4$) C, H, N. The glycolate salt, mp 172–174° dec (*Anal.* ($C_{27}H_{27}NO \cdot C_2H_4O_3$) C, H, N), was found to be approx 3 times more soluble (1:300) than the fumarate salt, which is more soluble than that of the other isomer. Admixture of the respective salts or the free bases of the isomers gave mp depression. Isomer purity was assayed by phase solubility measurements and both were found to be over 98.5% pure. They were also assayed by tlc on GF7/hydroxide plates using EtOH–C_6H_6, 1:19 satd with NH_3.

α-Fluoren-9-yl-*p*-tolyl)-2-piperidineethanol (1). Hydrogenation of 5 over Pd/C in DMF gave 1, mp 158–159°. *Anal.* ($C_{27}H_{29}NO$) C, H, N. This product probably represents one of the 2 diastereoisomers.

Acknowledgments. We acknowledge with appreciation the cooperation of our colleagues in the Biochemistry Department, Drs. T. R. Blohm and R. D. MacKenzie and associates. We derived substantial help from Dr. E. R. Andrews and D. L. Wenstrup, who attempted scale-up of the picolyllithium route and from L. Allen, L. A. Doerle, and J. G. Hetteberg, who worked on the scale-up by the 2,3,4,5-tetrahydropyridine–magnesium methyl carbonate route. We are also indebted to Drs. H. J. Keily, D. H. Gustafson, M. J. Gordon and associates for microanalyses, spectra, phase solubility, and chromatographic data.

References

(1) E. M. Roberts, G. P. Claxton, and F. G. Fallon, U. S. Patents 3,306,895 and 3,316,272 (1967); *Chem. Abstr.*, **68**, 21849, 39486 (1968).
(2) E. M. Roberts, G. P. Claxton, and F. G. Fallon, U. S. Patent 3,481,929 (1969); *Chem. Abstr.*, **70**, 77794 (1969).
(3) R. D. MacKenzie, E. M. Auxier, J. G. Henderson, J. M. Steinbach, and T. R. Blohm, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **29**, 647 (1970).
(4) R. D. MacKenzie, T. R. Blohm, E. M. Auxier, J. G. Henderson, and J. M. Steinbach, *Proc. Soc. Exp. Biol. Med.*, **137**, 662 (1971).
(5) R. D. MacKenzie, T. R. Blohm, J. M. Steinbach, and L. M. Lancaster, *Biochem. Pharmacol.*, in press.
(6) R. D. MacKenzie, T. R. Blohm, and E. M. Auxier, *Amer. J. Clin. Pa hol.*, **55**, 551 (1971).
(7) N. N. Goldberg, L. B. Barkley, and R. Levine, *J. Amer. Chem. Soc.*, **73**, 4301 (1951), and subsequent papers.
(8) K. Ziegler and W. Schäfer, *Justus Liebigs Ann. Chem.*, **511**, 101 (1934).
(9) M. Stiles and H. L. Finkbeiner, *J. Amer. Chem. Soc.*, **81**, 505 (1959).
(10) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," Wiley, New York, N. Y., 1967, pp 631–633.
(11) M. Stiles, *J. Amer. Chem. Soc.*, **81**, 2598 (1959).
(12) C. Schöpf, A. Komzak, F. Braun, and E. Jacobi, *Justus Liebigs Ann. Chem.*, **559**, 1 (1948).
(13) C. Schöpf, H. Arm, and H. Krimm, *Chem. Ber.*, **84**, 690 (1951).
(14) C. Schöpf, H. Koop, and G. Werner, *ibid.*, **93**, 2457 (1960).
(15) C. Schöpf, *Angew. Chem.*, **61**, 31 (1949).
(16) C. Schöpf, F. Braun, K. Burkhardt, G. Dummer, and H. Müller, *Justus Liebigs Ann. Chem.*, **626**, 123 (1959).
(17) J. van Noordwijk, J. J. Mellink, B. J. Visser, and J. H. Wisse, *Recl. Trav. Chim. Pays-Bas*, **82**, 763 (1963).
(18) J. H. Wisse, H. de Klonia, and B. J. Visser, *ibid.*, **83**, 1265 (1964).
(19) J. H. Wisse, H. de Klonia, and B. J. Visser, *ibid.*, **85**, 865 (1966).
(20) C. Schöpf, F. Braun, and A. Komzak, *Chem. Ber.*, **89**, 1821 (1956).
(21) E. E. van Tamelen and G. G. Knapp, *J. Amer. Chem. Soc.*, 77, 1860 (1955).
(22) J. Thesing, S. Klüssendorf, P. Ballach, and H. Mayer, *Chem. Ber.*, **88**, 1295 (1955).
(23) D. W. Fuhlhage and C. A. VanderWerf, *J. Amer. Chem. Soc.*, **80**, 6249 (1958).
(24) M. Kuna and M. J. Kopac, *Ann. N. Y. Acad. Sci.*, **58**, 261 (1954).
(25) R. E. Allen, E. L. Schumann, W. C. Day, and M. G. Van

Campen, Jr., J. Amer. Chem. Soc., 80, 591 (1958).
(26) H. B. Wright, D. A. Dunnigan, and U. Biermacher, J. Med. Chem., 7, 113 (1964).
(27) R. H. Cornforth, J. W. Cornforth, and G. Popjak, Tetrahedron, 18, 1351 (1962).
(28) Reference 10, pp 144-147.

Synthesis of New 2-Alkylamino-1,4-naphthoquinones as Inhibitors of Coenzyme Q and as Antimalarials†

Thomas H. Porter, Frederick S. Skelton, Christine M. Bowman, and Karl Folkers*

Institute for Biomedical Research, The University of Texas at Austin, Austin, Texas 78712. *Received December 11, 1971*

As based on the essentiality of coenzyme Q_8 in the metabolism of *Plasmodium*, new lipoidal 1,4-naphthoquinones have been synthesized as potential inhibitors of the biosynthesis and/or function of coenzyme Q_8 in the metabolism of *Plasmodium* and as potential antimalarials. Eight new 2-alkylamino-1,4-naphthoquinones have been synthesized and tested for antimalarial activity against *Plasmodium berghei* in the mouse. Three compounds (one new, and two previously prepared derivatives) showed evidence of activity by $T - C$ values ranging from 1.3 to 3.7 at 640 mg/kg dose level without toxicity by antimalarial assay against *P. berghei* in the mouse. The 2-alkylamino-1,4-naphthoquinones, represented in the assays by 7 of the 8 new compounds and 3 previously prepared derivatives, showed no significant inhibition of either the NADH- or succinoxidase enzyme systems. In contrast, our recently prepared 7-alkyl-6-hydroxy-5,8-quinolinequinones and the isomeric 6-alkyl-7-hydroxy-5,8-quinolinequinones were highly potent inhibitors.[1,2] The former derivatives were found to be powerful inhibitors of both *in vitro* mitochondrial enzyme systems, whereas the latter compounds were inhibitory only in succinoxidase.[1] Similarly a number of 6-alkylamino-5,8-quinolinequinones were inhibitory to both the NADH-oxidase and the succinoxidase systems, and inhibition of the NADH-oxidase system could be completely reversed by CoQ_{10}.[3]

The previous research which constitutes the background for this work has been described.[1-3] Recently, a series of new 7-alkyl-6-hydroxy-5,8-quinolinequinones and 6-alkyl-7-hydroxy-5,8-quinolinequinones have been synthesized and tested for antimalarial activity against *Plasmodium berghei* in the mouse.[2] Antimalarial activity accompanied by no observable toxicity at the highest levels tested was demonstrated for a number of these lipoidal 5,8-quinolinequinones. Many of these compounds were also evaluated in mitochondrial NADH- and succinoxidase systems for inhibition of coenzyme Q, and these *in vitro* data were compared with the data obtained from testing, *in vivo*, these compounds against *P. berghei* in the mouse.[1,2]

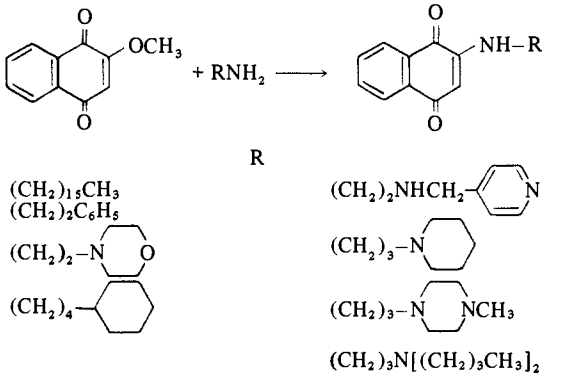
A series of 15 6-alkylamino-5,8-quinolinequinones (and one 7-alkylaminomethyl-6-hydroxy-5,8-quinolinequinone) have also been synthesized and tested for antimalarial activity against *P. berghei* in the mouse.[3] Ten of the total of 16 compounds showed definite antimalarial activity against *P. berghei* in the mouse. Three of the compounds met the arbitrary criterion of effectiveness to be declared "active," and none of these 3 showed toxicity at the highest level tested (640 mg/kg). Representative compounds were evaluated in mitochondrial NADH- and succinoxidase systems for inhibition of coenzyme Q. Seven 6-alkylamino-5,8-quinolinequinones were highly inhibitory to both NADH- and succinoxidase systems.

The syntheses and biological activities of 8 new 2-alkylamino-1,4-naphthoquinones are described herein. In view of the promising antimalarial activity of our newly synthesized 5,8-quinolinequinones, it was of current interest to prepare analogous naphthoquinone derivatives, *i.e.*, 2-alkylamino-1,4-naphthoquinones with alkyl side chains designed to increase the lipoidal character of the molecule and with alkyl side chains containing varying numbers of heteroatoms in an attempt to design molecules which could function as antimetabolites of the highly lipoidal coenzyme Q_8 of *Plasmodium* species.

†Coenzyme Q. 147.

Organic Syntheses. The synthesis of the 8 new 2-alkylamino-1,4-naphthoquinones was accomplished by treating 2-methoxy-1,4-naphthoquinone[4] in EtOH with the appropriate alkylamine as indicated in Scheme I. The MeO group

Scheme I



of 2-methoxy-1,4-naphthoquinone could be replaced by an amino group upon direct interaction with the appropriate alkylamine.[4,5] Generally, the amines reacted very readily with 2-methoxy-1,4-naphthoquinone, particularly the short chain aliphatic primary amines. Each of the 2-alkylamino-1,4-naphthoquinones was a red or orange crystalline substance with a sharp melting point (Table I).

Results of Antimalarial Assays. These compounds were tested for antimalarial activity against *P. berghei* in mice.[7] A single dose at the desired level was given sc 72 hr after the mice were infected with *P. berghei*. A minimum mean survival time of 13.0 days was required for the compound to be declared "active;" control mice exhibited a mean survival time of 6.2 days. Mice living 60 days or more after treatment were considered as cured.

One of the 8 new 1,4-naphthoquinones (**9**) showed definite activity ($T - C = 3.1$ at 640 mg/kg) in the *in vivo* antimalarial test against *P. berghei* in the mouse by procedures devised by Rane.[7] However, this compound was not declared