# Antiinflammatory Activity of Isomeric Phenylnaphthaleneacetic Acids

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The isomeric phenylnaphthaleneacetic acids were prepared and tested for antiinflammatory activity by the anti-UV-erythema method. High potency was exhibited by 4- and 5-phenyl-1-naphthaleneacetic acid and 5- and 6-phenyl-2-naphthaleneacetic acid. The results are discussed in terms of a hypothetical receptor site.

In an earlier report from these laboratories,[1] we described a series of 4- and 5-aryl-1-naphthaleneacetic acids which showed high potency as antiinflammatory agents. During the course of this work we had occassion to prepare all the isomeric phenylnaphthaleneacetic acids (with the exception of 8-phenyl-1-naphthaleneacetic acid) and wish to describe here the effect on potency caused by these structural variations.

**Chemistry.** The compounds described here were prepared by two general methods (see Scheme I). In method A, the appropriate phenylmethylnaphthalene[2-5] was treated with NBS to effect side-chain bromination, the bromomethyl compound treated with NaCN, and the resulting nitrile hydrolyzed with base to give the acetic acid. In the preparation of 1, 2-phenylnaphthalene was chloromethylated, and the sequence continued as described above. The identity of the melting point of 1 with that reported in the literature[6] for 1 prepared in an unambiguous manner confirms the position of chloromethylation. With this isomer, basic hydrolysis of the nitrile proceeded only as far as the amide. Treatment of the amide with butyl nitrite gave the desired acetic acid.

In method B the appropriate phenyl-substituted tetralone was allowed to react with ethyl bromoacetate under Reformatsky conditions, and the resulting dihydronaphthalene aromatized with sulfur. Basic hydrolysis of the ester gave the acetic acid.

In general intermediates were not extensively purified but were carried through the sequence to the acetic acid stage where acidic products were easily separated. The crude acids were then purified by crystallization or chromatography on silica gel.

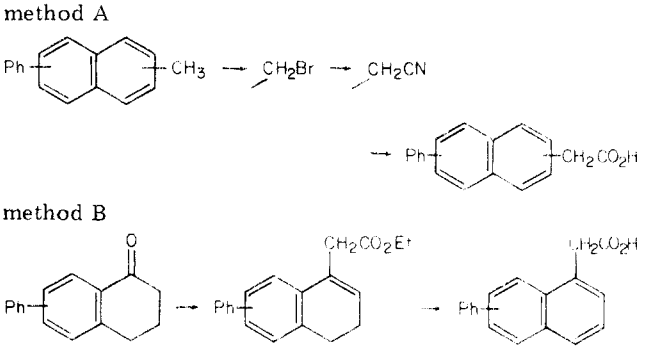
Tables I and II list the compounds prepared together with physical data and biological activity.

**Pharmacology.** Compounds were screened for their ability to suppress the erythema developing in albino guinea pig skin 2 h after a standard exposure to ultraviolet irradiation using Winder's modification[7] of Wilhelmi's method.[8] All agents were administered by gavage to depilated albino guinea pigs. Responses to treatment on an all-or-none basis were compared for test drug ($N = 5$) and the reference dose of phenylbutazone ($N = 10$) or vehicle ($N = 10$). The significance of treatment contrasts was determined by reference to tables of ready-computed probabilities.[9] Compounds significantly more active than vehicle ($0.05 > p$) were then tested at one-half the previous dose until a dose was reached where the response was significantly less than that of the reference level of phenylbutazone ($0.05 > p$).

## Discussion

Scherrer, Short, and Winder, considering *N*-arylanthranilic acids, discussed a hypothetical receptor site for nonsteroidal antiinflammatory agents.[10] Shen,[11] in considering indomethacin derivatives, discussed a similar receptor. The essential features of these receptor models were a large, flat area where an aromatic nucleus could be accommodated, a trough where an out-of-plane group

Scheme I




could be accommodated, and across the flat area, roughly "para" to the trough, a cationic site to which an acid anion could be bound. Most of the arylacetic acids which have been reported to have antiinflammatory activity seem to fit this qualitative description of the receptor.[12] Recently Scherrer[13] has discussed this same receptor in connection with prostaglandin synthetase inhibition by nonsteroidal antiinflammatory agents.

The present series of phenylnaphthaleneacetic acids, where a relatively large number of isomers are possible, gives a dramatic indication of the structural requirements necessary for high activity. Table I shows the high potency exhibited by 4- and 5-phenyl-1-naphthaleneacetic acid, where the phenyl and the acetic acid side chain have an approximate "para" relationship to each other. In Table II, although the potency is distinctly less, the high potency resides in two isomers, the 5- and 6-phenyl-2-naphthaleneacetic acids. Again a rough "para" relationship exists. As the 5-phenyl derivative in the 1-naphthaleneacetic acid series is ca. 30 times more potent than the corresponding isomer in the 2-naphthaleneacetic acid series, the overall configuration represented by 4- and 5-phenyl-1-naphthaleneacetic acid is apparently preferred for optimal activity.

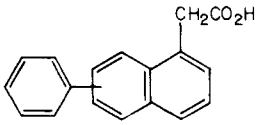
During the course of this work, we were unsuccessful in preparing 8-phenyl-1-naphthaleneacetic acid. After the completion of this work a report appeared describing its preparation.[14] Based on the ideas developed here, we would expect this isomer to show little or no activity as an antiinflammatory agent.

While the proper size and shape of a molecule is assumed to be a necessary requirement for activity, it is not a sufficient one. The compound must also reach the receptor in adequate concentrations. Therefore such physical parameters as $pK_a$, partition coefficient, rate of adsorption, and rate of metabolism are important. If one assumes that in this series of isomers, variations in these physical parameters are small (see Tables I and II), then the phenomenon observed here is indeed one of molecular shape.

## Experimental Section

**Method A.** Experimental details for this sequence have been

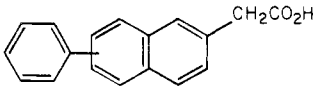
Table I. Phenyl-1-naphthaleneacetic Acids[a]



| Compd no. | Isomer | Min effective antiinflam dose, mg/kg[b] | Mp, °C[c] | Recrystn solvent | p$K_a$[d] | Apparent partition[e] coeff | Method of prepn |
|---|---|---|---|---|---|---|---|
| 1 | 2-$C_6H_5$ | >50[f] | 190-191.5 | $C_6H_6$ | 6.9 | 17 | A |
| 2 | 3-$C_6H_5$ | 50 | 164.5-166 | $C_6H_6$-hexane | 6.7 | 34 | B |
| 3 | 4-$C_6H_5$ | 0.1 | 120.5-121.5 | $C_6H_6$-hexane | 6.8 | 36 | A |
| 4 | 5-$C_6H_5$ | 0.1 | 180-182 | $C_6H_6$-hexane | 6.7 | 35 | A |
| 5 | 6-$C_6H_5$ | 25 | 201-203 | EtOH | 6.9 | 42 | A |
| 6 | 7-$C_6H_5$ | 100 | 152-154 | $C_6H_6$-cyclohexane | 6.9 | 33 | B |

[a] All compounds have the formula $C_{18}H_{14}O_2$ and analyzed within ±0.3% for C and H unless otherwise stated. [b] Preliminary screening estimates, except for compounds 3, 4, and 10 which are calculated from the results of a formal quantitative bioassay.[1] The minimum effective doses in this test of some reference agents are phenylbutazone, 5.3; indomethacin, 1.7; and meclofenamic acid, 0.4 mg/kg. [c] Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. [d] p$K_a$'s were determined by titration in 67% v/v of DMF-$H_2O$. [e] Compounds were partitioned between octanol-0.1 N pH 7 phosphate buffer and concentrations determined by UV absorption. [f] Insufficient sample for testing at the highest test level of 200 mg/kg.

Table II. Phenyl-2-naphthaleneacetic Acids[a]



| Compd no. | Isomer | Min effective antiinflam dose, mg/kg[b] | Mp, °C[c] | Recrystn solvent | p$K_a$[d] | Apparent partition[e] coeff | Method of prepn |
|---|---|---|---|---|---|---|---|
| 7 | 1-$C_6H_5$ | >200 | 157.5-159.5[h] | $C_6H_6$-hexane | 7.2 | 32 | A |
| 8 | 3-$C_6H_5$ | >200 | 145.5-147.5 | $C_6H_6$-hexane | 7.1 | 18 | A |
| 9 | 4-$C_6H_5$ | 100 | 138.5-140 | $C_6H_6$-cyclohexane | 7.0 | 28 | A |
| 10 | 5-$C_6H_5$ | 3.1 | 133.5-135.5 | Cyclohexane | 6.6 | 27 | A |
| 11 | 6-$C_6H_5$ | 3.1 | 207.5-209.5 | Chlorobenzene | 6.6 | 36 | A |
| 12 | 7-$C_6H_5$[g] | >50[f] | 206-208 | $C_6H_6$-EtOAc | 6.7 | 29 | A |
| 13 | 8-$C_6H_5$ | >100[f] | 140-142 | $C_6H_6$-hexane | 6.8 | 24 | A |

[a-f] See corresponding footnotes in Table I. [g] C: calcd, 82.42; found, 82.02. [h] Lit.[18] gives mp 158-159 °C.

described previously.[1,15] Chloromethylation conditions have also been reported.[1]

**2-Phenyl-1-naphthaleneacetamide.** A suspension of 16.3 g (0.067 mol) of crude 2-phenyl-1-naphthaleneacetonitrile in 100 mL of EtOH and 50 mL of $H_2O$ was treated with 20 g of KOH and heated at reflux overnight. After this time a solid was still present so the mixture was cooled and diluted with $H_2O$, and the solid was collected. Infrared analysis showed this to be 2-phenyl-1-naphthaleneacetamide.

**2-Phenyl-1-naphthaleneacetic Acid.** A suspension of 8.7 g (0.033 mol) of 2-phenyl-1-naphthaleneacetamide in 250 mL of dioxane was treated with HCl gas for 15 min, resulting in complete solution of the amide. Butyl nitrite (14 g, 0.13 mol) was then added dropwise over a 0.5-h period, and the solution then allowed to stir at room temperature for 2 h. The solution was then heated on a steam bath for 2 h and poured into $H_2O$. The solution was made basic with NaOH and extracted with $Et_2O$. Acidification with dilute HCl gave an oil which was extracted into $Et_2O$. Drying over $Na_2SO_4$ and removal of the $Et_2O$ under reduced pressure gave 4 g of the crude acid. Several recrystallizations from $C_6H_6$ gave 1.3 g of pure acid, mp 190–191.5 °C (lit.[6] gives mp 192–193 °C).

**Method B (Compounds 2 and 6).** A solution of 9.8 g (0.044 mol) of the appropriate phenyltetralone[16,17] and 8.2 g (0.049 mol) of ethyl bromoacetate in 100 mL of $Et_2O$ and 100 mL of $C_6H_6$ was treated with 20 g of Zn (20 mesh, acid washed) and a few crystals of $I_2$ and heated at reflux for 2 h. The solution was then decanted from the excess Zn and the Zn washed with $Et_2O$. The combined organic phases were washed with dilute HCl and then $H_2O$ until neutral. Drying over $Na_2SO_4$ and removal of the organic solvents under reduced pressure left an oil.

This was dissolved in 25 mL of $HCO_2H$ and heated on the steam bath for 5 min. The solution was diluted with $Et_2O$ and washed with $H_2O$, saturated $NaHCO_3$, and then $H_2O$ until neutral. Drying over $Na_2SO_4$ and removal of the solvent under reduced pressure left the dihydro derivative as an oil.

**Aromatization and Hydrolysis.** A mixture of 11.8 g (0.0405 mol) of the dihydro compound with 1.3 g (0.0405 mol) of sulfur was heated at approximately 220 °C for 1 h with stirring. Hydrogen sulfide was given off throughout the heating period.

The hot mixture was then poured into a solution of 10 g of KOH in 75 mL of EtOH and 25 mL of $H_2O$ and heated at reflux for 1 h. The solution was concentrated under reduced pressure and the residue taken up in $H_2O$. After washing with $Et_2O$, acidifying with dilute HCl caused the crude acid to precipitate. Several recrystallizations gave pure acid.

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# Synthesis of 5-Azacytidine-6-$^{13}C$ and -6-$^{14}C$

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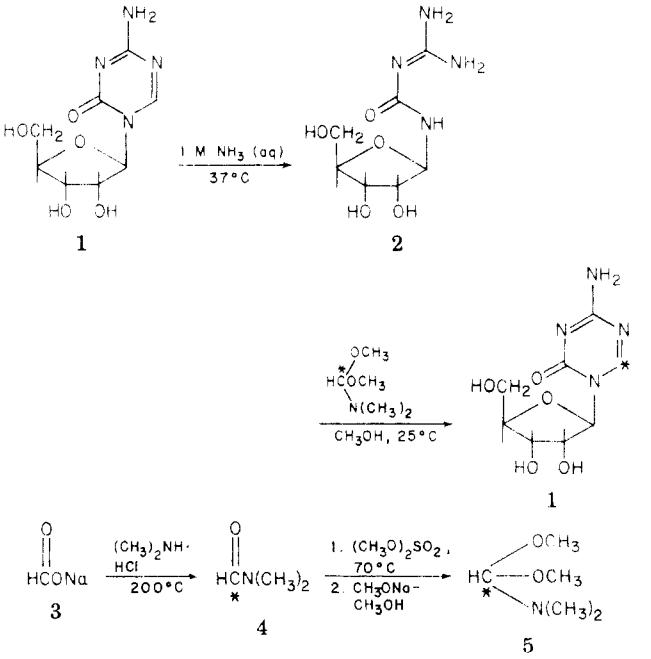
5-Azacytidine (1) labeled with $^{13}C$ or $^{14}C$ at the chemically labile C-6 position was synthesized. A method utilizing hydrolytic opening of the triazine ring followed by recyclization with dimethylformamide dimethyl acetal was used. Urinary and biliary excretion was measured in rabbits following intravenous doses of 1-4-$^{14}C$ and 1-6-$^{14}C$. Differences in recoveries of the dose from 4-$^{14}C$ and 6-$^{14}C$ demonstrate that ring cleavage of 1 with loss of the C-6 carbon represents a major metabolite route.

The nucleoside antimetabolite 5-azacytidine [4-amino-1-β-D-ribofuranosyl-*s*-triazin-2(1*H*)-one, NSC-102816, 1] is an effective antitumor[1,2] and antileukemic[3-5] agent. Chemically, 1 is known to be relatively unstable in aqueous solution[6,7] as compared to cytidine itself. The facile hydrolytic opening of the triazine ring of 1 at C-6 involves an intermediate which loses C-6 as formate ion and yields the guanylurea derivative **2**.[6,7] Metabolically, a scheme involving cleavage of 1 at C-6 was postulated on the basis of the metabolites isolated in 1-treated mouse urine.[8,9] Since both chemical and metabolic evidence indicates that ring cleavage of 1 is a facile process, the in vivo role of this process may contribute to its overall biological activity.

It has been suggested that incorporation of 1 into RNA of mouse liver represents the mechanism of action of this compound,[10] which was demonstrated utilizing 1-4-$^{14}C$. However, the use of 1 labeled with $^{14}C$ at the C-4 position alone does not allow one to assess the significance of the ring cleavage process. If ring cleavage following RNA incorporation is important in the molecular mechanism of its pharmacological activity, then 1 specifically labeled with $^{13}C$ and $^{14}C$ at C-6 should represent a valuable probe for the determination of its biological disposition.

**Synthesis.** The introduction of the $^{13}C$ and $^{14}C$ atoms into 1 is summarized in Scheme I and involves the formation of a triazine ring by condensation of 1-β-D-ribofuranosyl-3-guanylurea (**2**) with $^{13}C$- or $^{14}C$-labeled dimethylformamide dimethyl acetal (**5**). Ring closure occurred under mild conditions with 1 precipitating as formed. Compound **2** was obtained by mild alkaline hydrolysis of 1,[6] and $^{13}C$- or $^{14}C$-labeled acetal **5** was synthesized by a three-step sequence from sodium formate-$^{13}C$ or -$^{14}C$ (**3**). First the $^{13}C$- or $^{14}C$-labeled sodium formate was allowed to react with dimethylamine hydrochloride. The resulting dimethylformamide (**4**) was distilled as a hydrate[11] which had to be chemically dried

Scheme I




or formation of its dimethyl sulfate addition complex was inhibited. The complex, once formed, however, was immediately converted to **5** upon addition to sodium methoxide in methanol.[12] The identity of the synthetic 1 was substantiated by comparison of the carbon-13 NMR spectrum, mass spectrum, IR spectrum, melting point, and GC properties with those of an authentic sample.

**Biological Results.** Studies of the difference in the in vivo excretion pattern in urine and in the bile of 1-4-$^{14}C$ and 1-6-$^{14}C$ were carried out in six male New Zealand white rabbits. Doses of 15 mg/kg of 1-4-$^{14}C$ or 1-6-$^{14}C$ were