Notes

Nonsteroidal Antiinflammatory Agents. 2. Synthesis and Biological Activity of 2-Chloroindolecarboxylic Acids

Aldo Andreani,* Daniela Bonazzi, Mirella Rambaldi, Adriano Guarnieri,

Istituto di Chimica Farmaceutica e Tossicologica, Universita di Bologna

Franco Andreani,

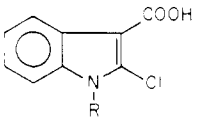
Istituto di Chimica degli Intermedi, Università di Bologna

Paola Strocchi, and Nicola Montanaro

Istituto di Farmacologia, Università di Bologna, Italy. *Received December 28, 1976*

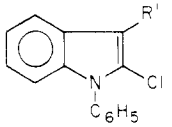
The Vilsmeier and the Arndt–Eistert reactions have been employed for the synthesis of 1-phenyl-2-chloroindole-3-acetic acid (**4**). The antiinflammatory activity of 2-chloroindole-3-carboxylic acid (**1**), 1-methyl-2-chloroindole-3-carboxylic acid (**2**), 1-phenyl-2-chloroindole-3-carboxylic acid (**3**), and **4** was compared with the activity of indomethacin in the carrageenin rat edema. The best results are given by compounds **1** and **2** bearing H or CH_3 at position 1 and COOH at position 3.

In a previous paper[1] the synthesis of the following 2-chloroindole-3-carboxylic acids has been reported.



1, R = H
2, R = CH_3
3, R = C_6H_5

We now wish to describe the synthesis of 1-phenyl-2-chloroindole-3-acetic acid (**4**) and the antiinflammatory activity of compounds **1**–**4** in the carrageenin edema test in order to establish (1) the influence of the substituent (H, CH_3, C_6H_5) in position 1 when there is a carboxyl group in position 3, and (2) the difference in activity between a 3-carboxylic acid and a 3-acetic acid when the same group (C_6H_5) is present in position 1 of an indole.



4, R' = CH_2COOH
5, R' = CHO
6, R' = COCl
7, R' = $COCHN_2$
8, R' = CH_2OH
9, R' = CH_2Cl
10, R' = CH_2CN
11, R' = CH_2CONH_2

Chemistry. For the synthesis of 1-phenyl-2-chloroindole-3-acetic acid (**4**), 1-phenyl-2-chloroindole-3-carboxylic acid (**3**) was used as the starting material, to which the well-known Arndt–Eistert[2] reaction was applied (**3** → **6** → **7** → **11** → **4**). The structure of **4** was confirmed by synthesizing the compound from the corresponding nitrile **10** (**5** → **8** → **9** → **10**); the hydrolysis of **10** proceeds through the amide **11** which has, in turn, been obtained from the acid **4**. The Vilsmeier reaction on 1-phenyl-2-indolinone for the synthesis of 1-phenyl-2-chloro-3-formylindole (**5**) was already described by one of us.[1] In the Experimental Section we report a modified method which enables us to obtain **5** (free from 1-phenyl-2-chloro-3,7-diformylindole[1]) with an improved yield. In addition, the procedure for the synthesis of 1-phenyl-2-chloroindole-3-carboxylic acid[1] (**3**) has also been simplified. The IR and ^1H NMR spectroscopic data of the new compounds are in agreement with the assigned structures.

Conclusion

Antiinflammatory activity of **1**, **2**, **3**, and **4**, at all doses employed, appears significantly lower than that of indomethacin (5 mg/kg os) at both observation times. Among the four 2-chloroindolecarboxylic acids tested, **2** shows the most potent and long-lasting activity and **1** has nearly the same pattern. A C_6H_5 group in position 1 produces a compound having little antiinflammatory activity, while the presence of H or CH_3 in the same position results in pharmacologically active compounds. Lengthening of the side chain in position 3 while retaining the same substituent (C_6H_5) in position 1 produces a compound (**4**) without any antiinflammatory activity.

Experimental Section

Chemistry. The melting points were taken on an Electrothermal apparatus (Model 1A-6304) and are uncorrected. The extracts were dried on anhydrous Na_2SO_4 and the organic solvents were evaporated under vacuum. Bakerflex (silica gel IB2-F) or Merck (Kieselgel 60-F254) plates were used for TLC; petroleum ether (bp 60–80 °C)–acetone mixtures were employed in various proportions as eluents. For column chromatography Kieselgel 60 (Merck) was used, activated at 120 °C for 2 h, in the proportion of 30 g per gram of substance; the eluent was a mixture of petroleum ether (bp 60–80 °C)–acetone (80:20).

1-Phenyl-2-chloro-3-formylindole (5).[1] The Vilsmeier reagent was prepared by adding, at 5 °C under nitrogen, 20 mL of $POCl_3$ (33.5 g, 218.5 mmol) to a stirred solution of DMF (20 mL, 18.88 g, 258.3 mmol) in $CHCl_3$ (20 mL). To this reagent was added, in the same conditions, 10 g of 1-phenyl-2-indolinone[3,4] (47.8 mmol) dissolved in 50 mL of $CHCl_3$ containing 10 mL of pyridine. After 48 h at room temperature, the mixture was poured onto ice. The chloroform layer was separated and the aqueous phase was extracted three times with $CHCl_3$. The combined extracts gave a solid residue consisting mainly of **5**, purifiable with a single crystallization from ethanol: mp 139–140 °C (10.4 g, 85%).

1-Phenyl-2-chloroindole-3-carboxylic Acid (3).[1] The method already described[1] was simplified as follows. **5** (2 g, 7.8

mmol) was dissolved in 200 mL of acetone; 3 g of $KMnO_4$ (18.9 mmol), dissolved in 60 mL of water, was added. The mixture was stirred for 6 h at room temperature; then it was decolored with 10% H_2O_2, filtered, evaporated, and acidified. The precipitate, pure on TLC, was crystallized from ethanol: mp 225 °C (1.9 g, 90%).

1-Phenyl-2-chloro-3-chloroformylindole (6). Dry **3** (4 g, 14.7 mmol) was treated with 50 mL of $SOCl_2$ (81.9 g, 688.4 mmol). The mixture was refluxed for 8 h and the excess $SOCl_2$ was removed under vacuum. The residue (**6**) was pure on TLC and the yield was practically quantitative. An analytical sample was crystallized from petroleum ether (bp 60–80 °C): mp 105–106 °C. Anal. ($C_{15}H_9Cl_2NO$) C, H, N.

1-Phenyl-2-chloroindole 3-Diazo Ketone (7). An ethereal solution of diazomethane (obtained from 10 g of nitrosomethylurea[5]) was added at 0 °C to a stirred solution of the crude chloride **6** in 150 mL of anhydrous ether. After 24 h at room temperature, 3 g of pure **7** was collected: mp 164–165 °C. After partial evaporation 1.2 g was obtained in microcrystalline form (total 4.2 g, 96.5%, calculated on **3**). Anal. ($C_{16}H_{10}ClN_3O$) C, H, N.

1-Phenyl-2-chloroindole-3-acetamide (11 from 7). **7** (4 g, 13.5 mmol) was dissolved in 100 mL of acetone. Under reflux 50 mL of 32% NH_4OH and 6 mL of water containing 0.6 g of $AgNO_3$ (3.5 mmol) were slowly added. Reflux was maintained and the reaction was followed by TLC. The mixture was cooled after about 1 h, filtered, and evaporated. The residue was crystallized from dilute ethanol: mp 140–142 °C (3.4 g, 88.3%). Anal. ($C_{16}H_{13}ClN_2O$) C, H, N.

1-Phenyl-2-chloroindole-3-acetic Acid (4). **11** (2.5 g, 8.8 mmol) dissolved in methanol (200 mL) was treated under reflux with 20% KOH (100 mL, 20 g, 356.4 mmol). The reaction was followed by TLC and after about 5 h the mixture was cooled, poured into ice containing 50 mL of concentrated HCl, and extracted with ether. The product was purified by column chromatography: mp 136–138 °C (1.8 g, 70%). An analytical sample was crystallized from petroleum ether (bp 60–80 °C): mp 138–140 °C. Anal. ($C_{16}H_{12}ClNO_2$) C, H, N.

1-Phenyl-2-chloro-3-hydroxymethylindole (8). **5** (6 g, 23.5 mmol) was dissolved in 200 mL of methanol and the solution was cooled. Under nitrogen and stirring 8 g (211.4 mmol) of $NaBH_4$ was added in small portions. After a few minutes at room temperature, the mixture was refluxed for 10 min, continuing with the nitrogen stream and stirring. The reaction was cooled, treated with water, and extracted with $CHCl_3$. The residue was crystallized from petroleum ether (bp 60–80 °C): mp 88–90 °C (4.8 g, 79.3%). Anal. ($C_{15}H_{12}ClNO$) C, H, N.

1-Phenyl-2-chloro-3-chloromethylindole (9). **8** (5 g, 19.4 mmol) was dissolved in 50 mL of benzene and treated, under cooling and stirring, with 5 g of PCl_5 (24.0 mmol). After 2 h at room temperature the mixture was refluxed for 1 h. Compound **9** was obtained after solvent elimination and it was used as such in the following reaction.

1-Phenyl-2-chloro-3-cyanomethylindole (10). The residue of the previous preparation (**9**) was dissolved in 100 mL of acetone. The solution was treated, under stirring at room temperature, with an aqueous solution (6 mL) of KCN (5 g, 76.8 mmol). After 3 h at room temperature the solvent was evaporated, water was added, and the mixture was extracted with $CHCl_3$. The residue was purified by column chromatography. After crystallization from ethanol, 2.3 g was obtained (40% calculated on **8**): mp 96–97 °C. Anal. ($C_{16}H_{11}ClN_2$) C, H, N.

1-Phenyl-2-chloroindole-3-acetamide (11 from 10). **10** (0.5 g, 1.9 mmol) was treated with H_2SO_4 (5 mL, 9.17 g, 93.5 mmol) for 5 min at 90 °C. The mixture was poured onto ice and extracted with ether. The residue was crystallized from dilute ethanol. The product was identical (melting point, IR, ^1H NMR) with that prepared from **7**.

1-Phenyl-2-chloroindole-3-acetamide (11 from 4). **4** (0.5 g, 1.7 mmol) was treated at room temperature with 5 mL of $SOCl_2$ (8.19 g, 68.8 mmol). After 15 min the mixture was evaporated and treated with 32% NH_4OH. The precipitate was collected and crystallized from dilute ethanol. The product obtained was identical (melting point, IR, ^1H NMR) with that prepared from **7** and from **10**.

Table I. Multiple Range Tests[a] for Comparisons of the Antiinflammatory Effect of 2-Chloroindolecarboxylic Acids

	Third Hour					
Dose	5 mg/kg os					
Compd	C[b]	4	3	1	2	I[b]
Mean value[c]	187.6	170.3	159.8	153.1	145.1	98.5
Dose	20 mg/kg os					
Compd	C	4	1	3	2	I
Mean value	187.6	165.9	161.7	149.4	146.5	98.5
Dose	40 mg/kg os					
Compd	C	3	1	2	4	I
Mean value	187.6	173.8	156.4	148.3	138.0	98.5
	Seventh Hour					
Dose	5 mg/kg os					
Compd	4	C	3	1	2	I
Mean value	200.9	178.8	151.4	143.4	133.3	106.8
Dose	20 mg/kg os					
Compd	4	C	1	2	3	I
Mean value	191.8	178.8	153.5	133.8	130.1	106.8
Dose	40 mg/kg os					
Compd	3	4	C	2	1	I
Mean value	201.1	186.4	178.8	150.7	132.1	106.8

[a] At 5% significance level (pooled standard error of means = 4.4). [b] I = indomethacin at 5 mg/kg os. C = controls. [c] Mean values of percent increases of foot volumes after subplanar carrageenin injection. Any two treatment means underscored by the same line are not significantly different.

Pharmacology. LD_{50} values after 72 h were determined by oral or intraperitoneal administration to groups of ten mice (Swiss strain male mice, weight 28–32 g); all the four compounds 1–4 showed an $LD_{50} > 500$ mg/kg.

Antiinflammatory activity of the compounds tested was evaluated by the inhibition of edema in the hind paw of rats in response to a subplantar injection of carrageenin.[6] Male rats (70) of the Wistar strain (weight 170–190 g) were used for the whole investigation. They were housed in an air-conditioned room, artificially illuminated from 7:00 a.m. to 7:00 p.m., and received commercial diet ad libitum. Room temperature was 22 ± 2 °C at a relative humidity of about 80%.

The tested compounds were suspended in 5% arabic gum and administered orally by stomach tube 1 h before the injection of 0.1 mL of a 1% suspension of carrageenin in 0.9% saline into the subplantar surface of the right hind foot of each rat.

The four 2-chloroindolecarboxylic acids 1–4 were tested at three dose levels (5, 20, and 40 mg/kg) using five animals for each dose. Two other groups of the same size, treated with indomethacin (5 mg/kg os) and 5% arabic gum alone, respectively, were included as controls. Foot volumes were measured with a mercury displacement plethysmograph immediately after and 3 and 7 h after the carrageenin injection, corresponding to the fourth and the eighth hour after the pharmacological treatment. We selected the two above-mentioned times of observation in order to evaluate the pharmacological activity of the compounds under examination against the edematous peak effect of carrageenin (third hour) and the duration of the effect itself (seventh hour).

For each animal, experimental data were represented by third and seventh hour percent increases in its foot volumes measured immediately after the carrageenin injection. Such results were submitted to analysis of variance and the mean values of all the groups were compared by means of Duncan's multiple range tests[7] separately for each dose level and each time of observation. Such tests included also comparison with 5% arabic gum and indomethacin mean values (see Table I).

Third Hour Results. All the compounds at 5 and 20 mg/kg have an antiinflammatory effect. Compound **2** is significantly more active than **4**, although it shows the same activity as 1 and

3. Compounds 1, 2, and 4 demonstrate antiinflammatory activity at 40 mg/kg while 3 does not differ from the control group. Such data point out that 2 shows a greater antiinflammatory activity compared to the other compounds at all doses tested.

Seventh Hour Results. Compounds 1, 2, and 3 show antiinflammatory activity at 5 and 20 mg/kg. At 40 mg/kg only 1 and 2 maintain their effect, while 3 causes an increased foot volume. Compound 4 is inactive at 5 mg/kg. The pharmacological data indicate that, in this series, compound 2 is the most active.

Acknowledgment. We are grateful to the Italian NRC (Consiglio Nazionale delle Ricerche) for its support on this project. We thank Professor M. Amorosa for his useful collaboration.

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Lysergic Acid Diethylamide. Photoelectron Ionization Potentials as Indices of Behavioral Activity

L. N. Domelsmith, Linda L. Munchausen, and K. N. Houk*[1]

Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803. *Received December 22, 1976*

The photoelectron spectrum of lysergic acid diethylamide (LSD) reveals five ionization potentials (IP's) between 7.25 and 9.75 eV arising from the aromatic (π) portion of the molecule and IP's of 8.4 eV arising from the tertiary amine and 8.5–9.0 and 9.1 eV arising from the amide group. Comparisons of the IP's of LSD, and of phenethylamines and tryptamines reported by us elsewhere, with activities of these compounds in rat and human behavioral tests show that increasing activity is paralleled by decreasing IP.

In 1959, Karreman et al.[2] suggested that the striking pharmacological activity of LSD and related compounds may arise from the ability of these compounds to act as charge-transfer or electron-transfer donors at the active site(s). Since that time, this hypothesis has been supported by the observation of charge-transfer complexes involving various drugs and electron acceptors,[3] as well as by approximate quantum mechanical calculations of orbital energies,[4] which usually increase as the activity of the drug increases. Purported correlations between the calculated orbital energies of a drug and various types of biological activity have been variously supported or denied over the last decade.[1,4,5]

The experimental quantities most closely related to calculated orbital energies are the ionization potentials of molecules. Koopmans' theorem provides the theoretical, but approximate, connection between experimental ionization potentials (IP's) and SCF-calculated molecular orbital energies (ϵ^{SCF}): $IP_i = -\epsilon_i^{SCF}$.[6] All calculations performed on molecules of moderate size are, of necessity, approximate, and, in any case, Koopmans' theorem is known to be deficient, especially in cases where orbitals of different types are compared. Photoelectron spectroscopy measures experimental ionization potentials of isolated molecules in the gas phase, where there is no interference from solvation and steric effects inherent in charge-transfer complexation studies. We report here the photoelectron spectrum of LSD and show how the experimental ionization potentials of this molecule are, indeed, lower than those of certain analogous, but less active, tryptamine and phenethylamine derivatives whose photoelectron spectra we have reported elsewhere.[7]

The photoelectron spectrum of LSD is shown in Figure 1, along with that of the simpler hallucinogenic analogue, *N,N*-dimethyltryptamine (DMT). Attempts to run lysergic acid amide and isolysergic acid amide were not successful because both samples underwent extensive decomposition at the elevated temperatures necessary for volatilization in our spectrometer. The spectra were recorded on a Perkin-Elmer PS-18 photoelectron spectrometer with He(I) source; the measurements were carried out on the molecules in the gas phase at elevated temperatures using xenon and argon as internal calibrants. To enable assignment of the various bands to ionizations arising from orbitals on the indole, tertiary amine, or amide moieties, we compare, in Figure 2, the vertical ionization potentials observed for LSD with those of models for the indole (*N,N*-dimethyltryptamine), tertiary amine and alkene (*N*-methyl-1,2,5,6-tetrahydropyridine), and amide (*N,N*-diethylisobutyramide) moieties present in LSD.

The lowest three π ionization potentials of *N,N*-dimethyltryptamine occur at 7.57, 8.22, and 9.54 eV, while the tertiary amine lone pair gives rise to an unresolved ionization near 8 eV.[7] An isolated trisubstituted alkene such as the 9,10 double bond in LSD would have an ionization potential of less than 9.37 eV, the value of the π IP of 1,2,5,6-tetrahydro-*N*-methylpyridine.[8] Conjugation of this π orbital with the π orbitals of the indole moiety of tryptamine will be appreciable, since these π systems are mutually twisted by only 11° in LSD.[9] This conjugation will have a relatively large effect on the lowest two DMT-like π ionization potentials, which arise from orbitals, π_1 and π_2, that are higher in energy than the trisubstituted alkene orbital. Thus, mixing of π_1 and π_2 with the alkene orbital will cause a destabilization of the two highest indole orbitals and a stabilization of the alkene orbital.[10] The mixing of the alkene π orbital with the third π orbital of the indole moiety will destabilize the alkene orbital and stabilize π_3. As shown in Figure 2, these considerations lead to assignment of the 7.25 ± 0.10, 8.04 ± 0.12, 8.54 ± 0.09, and 9.75 ± 0.10 eV ionization potentials to those arising from the orbitals of the 4-vinylindole π system. Three other low-energy ionizations are expected in the 7–9 eV regions of the spectrum. The tertiary amine lone pair ionization will be similar to that in the tetrahydropyridine model (8.67 eV).[8] This ionization must be in the intense region around 8.4 eV, which contains several ionization bands. *N,N*-Dimethylisobutyramide has carbonyl–nitrogen (π_{NCO}) and oxygen (n_O) ionization potentials of 8.80 and 9.14 eV, respectively. The former type is un-