SYNTHESIS AND ANTIINFLAMMATORY ACTIVITY OF 1-[2,6-D-DICHLOROPHENYL]INDOLIN-2-ONES

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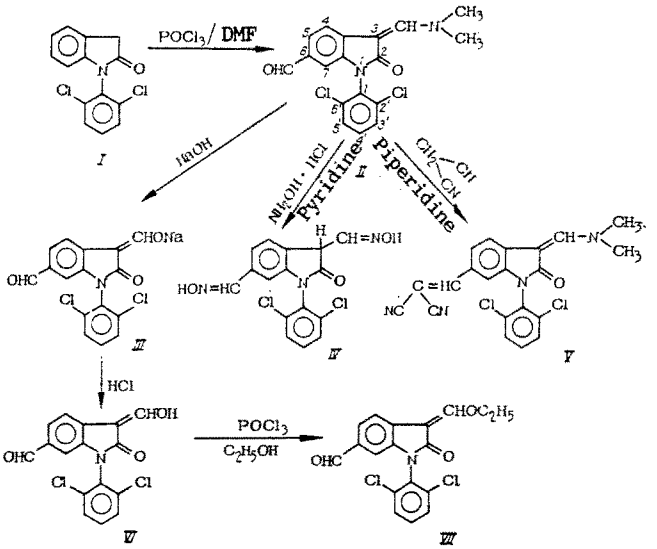
The search for antiinflammatory analogs of voltarene (sodium 2-(2,6-dichloroanilino) phenylacetate) has been carried out for the most part amongst derivatives of this acid (esters, amides, substitution products at the methylene group, etc.) [1, 3, 6-8]. Since voltarene has been found by us to undergo ready cyclization in the presence of acidic reagents to give 1-(2,6-dichlorophenyl)indolin-2-one (I), it was desirable to obtain some derivatives of this compound for testing for antiinflammatory activity.

The Vilsmaier reaction of N-phenylindolin-2-one is known [10] to give 50-60% of 1-phenyl-2-chloro-3-formylindole. In order to introduce functional groups into (I), we reacted it with the Vilsmaier reagent in chloroform at the boil for 6 h under the conditions described in [10]. The resulting compound (II), according to its elemental analysis and mass spectrum, differed from the starting material (I) in the presence of the formyl and dimethylaminomethylene groups. Since retention of the dimethylaminomethylene group is frequently observed following the decomposition of the Vilsmaier complex in pyrazoles [2] and indolinones [11], the dimethylaminomethylene group in (II) is evidently present in the 3-position of the indolinone ring.

The reactions of (I) and (II) are shown below:



On heating (II) with caustic alkali in aqueous alcohol, cleavage of the dimethylamino-group takes place with the formation of the sodium salt (III). Boiling (II) with hydroxylamine hydrochloride in the presence of pyridine also results in cleavage of the dimethylamino-group, but in this case the dioxime of the corresponding diformyl derivative (IV) is obtained. Reaction of (II) with malonodinitrile at the boil for 1 h in alcohol in the presence of piperidine results in condensation of the CH-acidic reagent with the formyl group

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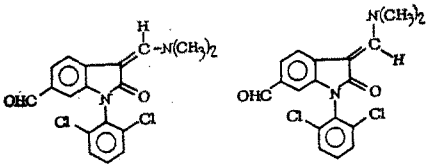
present in the aromatic protion of the molecule, the 3-dimethyl-aminomethylene group being retained (V). Acidification of the sodium salt (III) with hydrochloric acid affords (VI), which on boiling with phosphoryl chloride in absolute alcohol gave the ester (VII).

The ^1H NMR spectrum of (II) (solvent $CDCl_3$) confirmed the presence of both the dimethyl-aminomethylene and formyl groups, signals for the methyl group being present at 3.36-3.75 ppm, and a signal for the formyl proton at 9.84-9.85 ppm. It is noteworthy that in the spectrum of (II), doubling of the signals for the protons of the aromatic ring and the substituents ($-N(CH_3)_2$ and CHO) is seen. This suggests that this compound is a mixture of geometric isomers in a ratio of approximately 2:3.



I, Z-isomer

I, E-isomer

The spectrum of (II) obtained in $(CD_3)_2SO$ shows a marked change in the ratio of the Z and E isomers, the amount of one isomer being approximately 10%; compounds (V), (VI), and (VII), under similar conditions are present as pure isomers (their identification will be reported in a further publication).

Comparison of the ^1H NMR spectra of (I), (VI), and (VII) enables the position of the formyl substituent to be located unambiguously. In the spectrum of (I) in $(CH_3)_2SO$, signals for the 3', 4', and 5'-protons are well resolved as A_2B systems (7.78-7.73-7.57 ppm), together with the protons in the 4-7 positions, one of the signals for these protons being shifted towards higher field (6.38 ppm; boradened doublet). It appears that the dichlorophenyl radical departs from the plane of the molecule as a result of steric hindrance (the closeness of the protons at C_7 to the chlorine atom), thus screening the proton in the 7-position. On the basis of these considerations, the high-field signal in the aromatic proton region is assigned to the 7-proton.

In the ^1H NMR spectrum of (VI) and (VII) in $(CH_3)_2So$, the A_2B signals are retained (7.78-7.75-7.60 ppm) for the dichlorophenyl radical, thus excluding attachement of the formyl group to positions 3', 4', and 5'. The following signals correspond to the protons of the benzene moiety of the moelcule: a doublet (J 7,6 Hz) at δ 7.85 ppm, a quadruplet (J_1 7.6 Hz, J_2 1.4 Hz) at δ 7.71 ppm, and a doublet (J 1.4 Hz) at δ 6.82 ppm.

By analogy with the indolinone (I), the signal at highest field is assigned to the 7-proton. The type of splitting of this signal is in accordance with the presence of the formyl group in the 6-position.

The mass spectrum of (II) shows a strong peak for the molecular ion, mass number 360 (here and subsequently, the mass numbers for the ions containing the ^{35}Cl isotope are given). From the ratios of the intensities of the isotopic peaks in the molecular ion, the molecule contains two chlorine atoms. Breakdown occurs principally with the formation of the ions $[M—CH_3]^+$ (*m/z* 345), $[M—Cl]^+$ (*m/z* 325), $[M—N(CH_3)_2]^+$ (*m/z* 316), $[M—Cl—CH_3—N=CH_2]^+$ (*m/z* 302). In the spectrum of (II) and its derivatives (IV-VII), no peaks for ions formed by cleavage of the bond between the indolinone and aryl groups were seen, probably as a result of the high degree of conjugation of the rings.

The molecular mass of (IV), found by spectrometry, was 363. The principal breakdown route is removal of the substituents in the 3- and 6- positions: $[M—O]^+$ (*m/z* 347), $[M—OH]^+$ (*m/z* 346), $[M—H_2O]^+$ (*m/z* 345), $[M—NHOH]^+$ (*m/z* 331). In the mass spectrum of (V), in addition to a strong molecular ion peak with m/z 408, peaks are seen for ions formed by the stepwise removal of the dimethylamino-group and a chlorine atom: $[M—CH_3]^+$ (*m/z* 393), $[M—Cl]^+$ (*m/z* 373), $[M—N(CH_3)_2]^+$ (*m/z* 364), $[M—Cl—CH_3—N=CH_2]^+$ (*m/z* 330).

The strongest peak in the mass spectrum of (VI) is assigned to the molecular ion, m/z 333. The principal breakdown route is elimination of chlorine followed by removal of a molecule of carbon monoxide. The spectrum contains the ion peaks $[M—Cl]^+$ (*m/z* 298), $[M—Cl—CO]^+$ (*m/z* 270), $[M—Cl—2CO]^+$ (*m/z* 242)

The mass spectrum of (VII) shows a strong molecular ion peak with m/z 361, together with peaks for ions formed by the stepwise removal of the ethoxy-group and a chlorine atom: $[M—C_2H_4]$ (*m/z* 333), $[M—C_2H_5]^+$ (*m/z* 332), $[M—Cl]^+$ (*m/z* 326), $[M—CO—CH_3]^+$ (*m/z* 318), $[M—C_2H_4—Cl]^+$ (*m/z* 298).

TABLE 1. Antiinflammatory and Analgesic Activity of Diclofenac Sodium (VIII) Analogs

Compound	Antiinflammatory activity, ED_{50}, mg/kg		Analgesic activity, ED_{50}, mg/kg			Acute toxicity, LD_{50}, mg/kg
	carragenin edema in rats	peritonitis in mice	hot plate test	thermal irritation of tail	spasm	
IV	220	550	152	140	190	1200
V	20	14	25	12	10	38
VI	92	95	inact.	320	84	460
II	170	280	inact.	670	290	1200
III	inact.	inact.	inact.	inact.	inact.	560
I	160	170	500	450	240	1200
VIII	8	18	98	75	5	380

Note. "Inact" signifies inactive in doses of 30% of the LD_{50}.

EXPERIMENTAL CHEMICAL

Mass spectra were obtained on a Varian MAT-mass spectrometer (USA), with direct introduction of the sample into the source. The ionizing electron energy was 70 eV, and ionization chamber temperature 180°C. ^1H NMR spectra were recorded on a Varian XL-200 spectrometer (Switzerland). The purity of the compounds was checked by chromatography on Silufol UV-254 plates (Czezh SSR).

1,(2,6-Dichlorophenyl)indolin-2-one (I). A mixture of 100 g (0.31 mole) of N-chloroacetyl-2,6-dichlorophenylamine and 100 g (0.75 mole) of 99.6% anhydrous aluminum chloride was heated with stirring to 145-150°C, whereupon a vigorus reaction set it with copious evolution of hydrogen chloride and a spontaneous rise in temperature to 162-168°C. The melt was heated for a further 2 h, then poured into acidified water and ice. Stirring was continued with external cooling for 1 h, during which time the viscous, reddish-brown mass was converted into a solid. This was filtered off, and washed with water until neutral to give 71.88 g (81.3%) of (I), mp 128°C (from propan-2-ol). Found, %: C 60.33; H 3,11; N 4.93; Cl 25.96, $C_{14}H_9Cl_2ON$. Calculated, %: C 60.44; H 3.26; N 5.03, Cl 25.25.

1-(2,6-Dichlorophenyl)-3-(dimethylaminomethylene)-6-formylindolin-2-one (II). To the Vilsmaier complex obtained from 4.56 ml (0.05 mole) of phosphoryl chloride and 5 ml (0.06 mole) of DMF was added to 0°C over 30 min a solution of 2.78 g (0.01 mole) of (I) in 25 ml of chloroform. The mixture was stirred for 6 h, poured on to ice, neutralized with 1 N NaOH, extracted with chloroform, the extract dried over magnesium sulfate, and the solvent removed under reduced pressure. The oily yellow residue crystallized on chilling and trituration with light petroleum, and was filtered off to give 2.8 g (77.3%) of (II), mp 234-236°C (from abs. alcohol), Found, %: C 59.93; H 3.88; N 7.62; Cl 19.57; $C_{18}H_{14}N_2O_2Cl_2$. Calculated, %: C 59.85; H 3.87; N 7.75; Cl 19.65.

Sodium Salt of 1-(2,6-Dichlorophenyl)-3-hydroxymethylene-6-formylindolin-2-one (III). A mixture of 15 g (0.04 mole) of (II), 111 ml of 1 N NaOH, and 111 ml of ethanol was boiled with stirring for 2.5 h. The alcohol was evaporated, whereupon an oil separated from the aqueous alkaline solution. The aqueous layer was decanted off, and water was removed from the residue by adding benzene and distilling it off. The dried oily residue was dissolved in absolute alcohol, the inorganic matter filtered off, the solvent removed, and the oily residue triturated with benzene and acetone to give 11.3 g (73.5%) of (III), mp 300°C. Found, %: C 53.44; H 2.86; N 3.38; Cl 18.38. $C_{16}H_{10}Cl_2O_4NNa$. Calculated, %: C 53.93; H 2.24; N 3.93; 18.83.

1-(2,6-Dichlorophenyl)-3-hydroxymethylene-6-formylindolin-2-one (VI). Compound (III) (11.3 g, 0.03 mole) was dissolved in 100 ml of water, and 10% hydrochloric acid added to pH 4.0. The yellow solid which separated was filtered off and washed with water to give 9.8 g (95%) of (VI), mp 230-231°C (from ethyl acetate-alcohol, 1:1) C 57.39; H 2.68; N 4.06; Cl 21.21. $C_{16}H_9Cl_2O_3N$. Calculated, %: C 57.50; H 2.69; N 4.18; Cl 21.25.

1-(2,6-Dichlorophenyl)-3-ethoxymethylene-6-formylindolin-2-one (VII). To a solution of 2.85 g (0.085 mole) of (VI) in 20 ml of absolute ethanol was added at 60°C 1 ml of phosphoryl chloride, and the mixture heated for 2 h on the boiling water bath. The alcohol was removed, and the residue treated with water. The yellow solid was filtered off to give 1.18 g (38%) of (VIII), mp 183-185°C (from alcohol), Found, %: C 59.87; H 3.68; N 3.68; Cl 19.70. $C_{18}H_{13}Cl_2O_3N$. Calculated, %: C 59.67; H 3.58; N 3.86; Cl 19.59.

1-(2,6-Dichlorophenyl)-3,6-diformylindolin-2-one Dioxime (IV). A mixture of 2 g (0.005 mole) of (II), 10 ml of abs. ethanol, 10 ml of dry pyridine and 1 g (0.01 mole) of hydroxylamine hydrochloride was boiled for 2 h. The solvent was removed, and the oily residue triturated with water and filtered to give 1.9 g (84%) of (IV), mp 210-211°C (from propan-2-ol). Found, %: C 52.73; H 3.20; N 11.15; Cl 19.53. $C_{16}H_{11}Cl_2N_3O_3$. Calculated, %: C 52.75; H 3.04; N 11.53; Cl 19.49.

1-(2,6-Dichlorophenyl)-3-(dimethylaminomethylene)-6-(dicyanoethylene)-indolin-2-one (V). To a solution of 2 g (0.005 mole) of (II) in 140 ml of abs. ethanol was added a solution of 1 g (0.01 mole) of malonodinitrile in 5 ml of abs. alcohol and 2 ml of piperidine. The mixture was heated for 1 h, and the solid which separated was filtered off to give 0.83 g (36.5%) of (V), mp 259-261°C (from alcohol). Found, %: C 61.10; H 3.69; N 13.55; Cl 17.49; $C_{21}H_{14}Cl_2ON_4$. Calculated, %: C 61.61; H 3.44; N 13.68; Cl 17.34.

EXPERIMENTAL PHARMACOLOGICAL

The antiinflammatory activity of (I-VI) was examined in model paw edema in male rats weighing 120-140 g, measured by oncometry. The actute inflammatory process was induced by the subplantar introduction into the the right rear extremity of 0.1 ml of a 1% solution of carragenan [12]. Antiexudative effects were studied in model peritonitis in male mice weighing 23-25 g, induced by intraperitoneal administration of 0.25 ml of 1% acetic acid [4]. Analgesic activity was assessed in model pain reactions (spasm induced by the intraperitoneal administration of acetic acid [4] to male mice weighting 18-22 g). In addition, the effects of the compounds on the pain threshold in mice were examined using the hot plate [13] and thermal irritation of the tail [5] methods. In all the tests, the compounds were administered internally as 1% suspensions in starch mucilage in doses of 1, 3, 10, and 30% of the LD_{50}, which was calculated graphically by the method described in [9], the acute toxicites being determined in male mice weighing 16-18 g by the same mode of administration. The effective doses (ED_{50}) were found graphically, the activities of the compounds being compared with that of diclofenac sodium (VIII)

The tests showed that apart from (III) all the compounds possessed antiinflammatory and analgesic activity (Table 1).

In the carragenin model edema, the compounds are less active than (VIII) by factors of 2.5-28. The most active compound is (V). In the model peritonitis, (V) was 1.3 times as active as (VIII), whereas (I), (IV), and (VI) were 5-30 times less active. The analgesic activity of the test compounds in the lot plate test was 2-59 times less than that of (VIII). In thermal pain stimulus, the most active compound was (V), being 3.9-6.2 times as active as (VIII), the remaining compounds having 1/2-1/10 this activity. The acute toxicity figures showed that the most active compound (V) is highly toxic, i.e., it is markedly inferior to (VIII) in its therapeutic index. The remaining compounds were less toxic than (VIII).

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