Antiallergic Activity of 2-Nitroindan-1,3-diones

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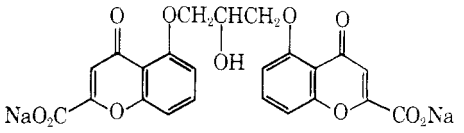
A number of substituted 2-nitroindan-1,3-diones are compared for antiallergic activity as measured by the homocytotropic antibody-antigen induced passive cutaneous anaphylaxis reaction in the rat.

In 1967 disodium cromoglycate (1) was first shown to be effective in the treatment of bronchial asthma.[1] It is still the only drug of its type that is used for the treatment of asthma, although a variety of compounds have been claimed, in the patent literature, as possessing a similar antiallergic activity. The antiallergic potential of a variety of substituted chromones related to disodium cromoglycate[2,3] and of some xanthone-2-carboxylic acids[4] has been assessed from their relative activities in the passive cutaneous anaphylaxis (PCA) reaction in the rat,[5,6] using rat homocytotropic antibody. Our finding that 2-nitroindan-1,3-dione (2) was active in this test led to the synthesis of a variety of substituted 2-nitroindan-1,3-diones. This paper describes the synthesis and activities in the rat PCA test of some of these compounds.



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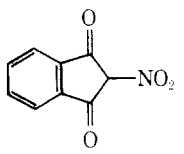

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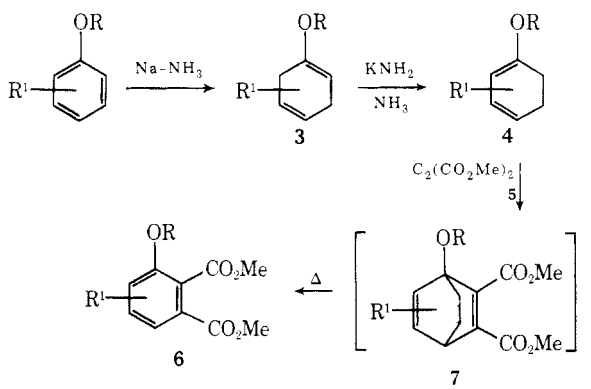
Chemistry. Synthesis of the 1,3-indandione nucleus has been undertaken by three routes (Schemes II → IV inclusive), the method of choice being dependent on the substitution required. All three procedures necessitate appropriately substituted phthalate precursors which have been obtained through a variety of synthetic methods.

The ready availability of the two mononitrophthalic acids lends itself to the facile introduction of simple functional groups such as halogen[7] and phenyl[8] but less easily to hydroxylated products[9] from which alkyloxyphthalates may be derived. A more convenient route to 3-alkyloxyphthalates is outlined below (Scheme I).

Scheme I



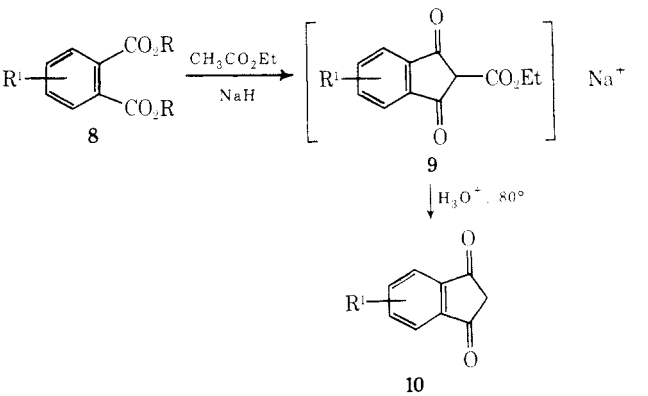
Reduction of alkyloxybenzenes with sodium in liquid ammonia has been shown to lead preferentially to the 2,5-dihydro product 3 with smaller amounts of 2,3-dihydro isomer 4.[10] Treatment of this mixture with potassium amide then effects isomerization to the conjugated diene 4 by way of a double bond shift at C-1.[11] Cyclization of 4 with dimethyl acetylenedicarboxylate (5) as described by Birch and Hextall[12] results in excellent yields of the substituted phthalic ester 6.

We have found that potassium amide in liquid ammonia does not always effect complete conjugation, but that as a general rule mixtures containing greater than 40% of the conjugated isomer will still result in high yields of the aromatized adduct. Presumably under the exothermic reaction conditions thermal isomerization of 3 to 4 occurs. Final thermolysis at 200° over 1 hr completed elimination of ethylene, the bicyclic adduct 7 never being isolated.

A similar route to the preparation of dihydrophthalates is provided by the Diels–Alder reaction of 1,3-dienes, many of which are commercially available, with acetylenedicarboxylic acid or more commonly its dimethyl ester.[13] Dialkyl, aryl alkyl, monoalkyl, and monoaryl dihydrophthalates have been prepared in this manner; all readily aromatize by aeration at 200° over 1 hr in the presence of 10% palladinized charcoal. The method, however, has the disadvantage that with complex dienes (*e.g.*, 1-phenylpenta-2,4-diene) extensive polymeriation and/or isomerization can ensue, resulting in intangible products.

The most versatile method for converting the phthalic esters to 1,3-indandiones is by Claisen condensation of the esters with ethyl acetate in the presence of sodium or its hydride[14] (Scheme II). The initially formed 2-carboethoxy sodium salts (9) spontaneously decarboxylate to indandiones (10) on dilute acid hydrolysis.

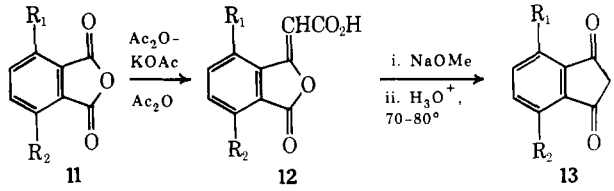
Scheme II



R = Me, Et; R^1 = alkyl, aryl, alkyloxy, aryloxy

For those phthalates substituted in both the 3 and 6 positions the Claisen condensation was unsatisfactory. With these examples condensation of the appropriate anhydrides (11) with acetic anhydride in the presence of anhydrous potassium acetate (Perkin reaction)[15] gave good yields of the phthalidylideneacetic acids (12). These smoothly convert to the diones (13) on alkaline rearrangement followed by decarboxylation at 80° in the presence of acid (Scheme III).

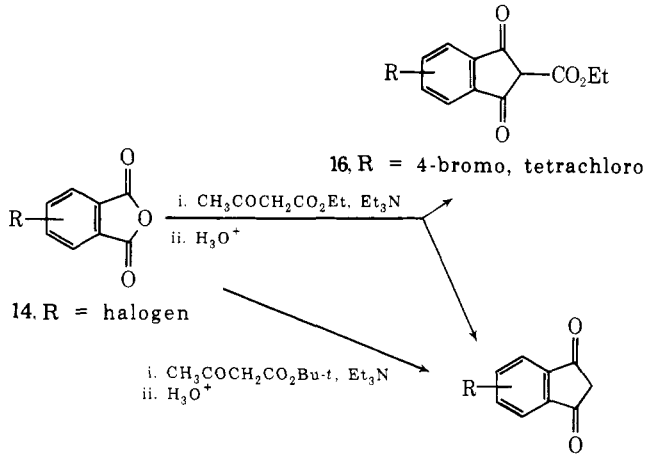
Scheme III



11 12 13

Phthalic anhydrides (14) have also been converted to 1,3-indandiones by reacting the anhydrides with ethyl acetoacetate in the presence of triethylamine[16] (Knoevenagel reaction) followed by acid work-up (Scheme IV). With certain anhydrides, notably 3-bromo and tetrachloro, intermediate esters of type 16 were isolated, these resisted decarboxylation. Replacement of ethyl acetoacetate by its *tert*-butyl ester, however, yielded the indandiones smoothly and in high yield. This method is generally recommended for halogenated derivatives.

Scheme IV



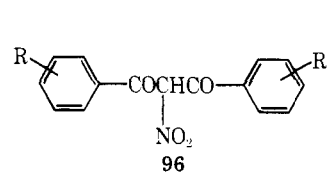
Without exception, the nitration of 1,3-indandiones was carried out between 0 and 20° in anhydrous ether using fuming nitric acid. In certain examples, where the diones were oxidized by the acid, temperatures <10° have been used (noted in Table IV). The tendency of 2-nitroindan-1,3-diones to form stable hydrates is reflected in certain of the elemental analyses, difficulty being experienced in removing the final half-molecule of water. Stable dihydrates are formed by some of the compounds (*e.g.*, 80 and 82), but under high vacuum drying over P_2O_5 rarely is more than the hemihydrate observed.

Results and Discussion

A series of 2-nitroindan-1,3-diones was screened for antiallergic activity as measured by the rat PCA test, and the results are reported in Table IV. There are too few compounds to enable rules to be made as to structural requirements for high activity. The parent unsubstituted 2-nitroindan-1,3-dione (2) showed an activity very close to that of disodium cromoglycate (1), and in general the substitutions reported in Table IV tended either to have little effect on the activity or to reduce it. The heavily substituted tetrachloro compound 95 showed no detectable activity. However, the two compounds carrying substituents at both positions 5 and 6 (87 and 88) showed a marked increase in activity.

Compounds in Table III which do not possess the nitro group at position 2 showed weak activity. Moreover, some compounds of general formula 96 showed no activity, and 97[17] was also inactive at doses up to 100 mg/kg sc.

The work of Friedmann[18] increased interest in bioisosteric groupings[19] and led us to replace one of the carbonyl



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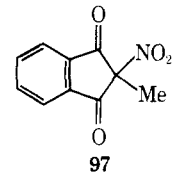

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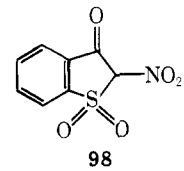
groups of nitroindandione by SO_2. The resulting compound (98) showed low activity.



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Experimental Section

Melting points were determined on a Kofler hot stage apparatus and are recorded uncorrected. The structures of all compounds were confirmed by ir and nmr spectroscopy, the latter as a solution in $CDCl_3$ for the diones and phthalate precursors or as a solution in DMSO-d_6 for the nitro derivatives. Where analogs are represented by elemental symbols (Table I–III) the results for these elements fall within ±0.4% of the calculated values.

Phthalate Precursors. The methods of preparation of the phthalate precursors are shown in Tables I and II. Dimethyl phthalate and tetrachloro- and 3-fluorophthalic anhydrides were commercially available.

Method I. Compounds 23, 28, and 33 were prepared by esterification of their anhydrides, the first two of which are obtainable commercially and the latter by dehydration of the diacid with thionyl chloride.

Method II. Alkyloxyphthalates (16–21, 34, 35, and 39) were prepared from the appropriate alkyloxybenzenes by the method of Birch and Hextall.[12] In general, the intermediate dihydro compounds were not analyzed but were assayed by nmr for their isomeric content after distillation at atmospheric pressure. Compounds 18, 19, and 21 did not solidify after distillation and were not purified. The nmr spectra, however, indicated <5–10% impurity.

Method III. 1-Phenylbuta-1,3-diene,[20] 1-vinylcyclohex-1-ene,[21,22] and 1-vinylcyclopent-1-ene[21,22] were synthesized by known methods, and their reaction with an equimolar amount of dimethyl acetylenedicarboxylate without solvent at the ambient reaction temperature afforded the dihydro aromatic adducts. Direct aromatization by aeration at 200° in the presence of 10% palladinized charcoal yielded the phthalic esters 22, 38, and 40, respectively.

Compounds 24, 30, and 32 were similarly prepared from commercially available dienes, although the increased volatility of these required cyclization in benzene at 80° in an autoclave. Dehydrogenation was affected as for 22, 38, and 40.

Compound 37 was prepared from 3,4-dihydronaphthalic anhydride[23] by esterification and aromatization as described above.

The bromophthalic acids 25 and 26 and their respective anhydrides 41 and 42 were prepared according to the method of Stephens.[7]

Compound 27 was synthesized from dimethyl 4-aminophthalate by the procedure of Butterworth, *et al.*[8]

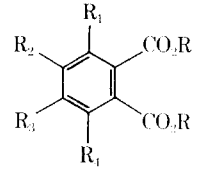
Compound 29 was prepared using the method of Wolinsky and Login.[24]

The acids 31 and 36 and the anhydride 44 were prepared by room temperature hydrolysis of the respective esters using 2.5 *N* sodium hydroxide. Compound 31 was converted to its anhydride, 43, with acetic anhydride.

Indan-1,3-diones. Physical data relating to substituted indan-1,3-diones (45–70) are listed in Table III along with the methods of preparation.

Irrespective of the method of preparation, all 1,3-indandiones were purified by recrystallization from either benzene or, less usually, acetone. Small amounts of insoluble matter isolated at this stage were identified as dimeric structures, in agreement with the known tendency of these compounds to form self-condensation products.[11] The noticeable increase in this insoluble fraction on prolonged acid treatment or use of temperatures above 80° invoked care during the decarboxylation stage. In general, temperatures in the range of 70–80° were used and of duration 7–15 min. Representative examples of each of the three general

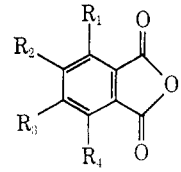
Table I. Intermediate Phthalate Esters and Acids



Compd	R	R_1	R_2	R_3	R_4	Mp, °C	Bp, °C (mm)	Formula	Analyses	Lit. mp, °C	Lit. bp, °C (mm)	Yield, %	Method of preparation[a]
16	Me	OMe	H	H	H	75–77 (MeOH)	122–134 (0.1)	$C_{11}H_{12}O_5$		77–77.5[12]	130–140 (1.0)[12]	73	II
17	Me	OEt	H	H	H	58 (MeOH)	152 (0.1)	$C_{12}H_{14}O_5$	C, H			36	II
18	Me	OPr-*n*	H	H	H		130–140 (0.5)	$C_{13}H_{16}O_5$	Crude			95	II
19	Me	OPr-*i*	H	H	H		217 (0.2)	$C_{13}H_{16}O_5$	Crude			81	II
20	Me	OBu-*n*	H	H	H	48–49 (MeOH)	170 (0.3)	$C_{14}H_{18}O_5$	C, H			52	II
21	Me	OBu-*i*	H	H	H		140–144 (0.1)	$C_{14}H_{18}O_5$	Crude			89	II
22	Me	Ph	H	H	H	96 (Et_2O–pet. ether)		$C_{16}H_{14}O_4$	C, H	94[8]		56	III
23	Et	Me	H	H	H		119 (0.5)	$C_{13}H_{16}O_4$	C, H			76	I
24	Me	Et	H	H	H		126 (1.2)	$C_{12}H_{14}O_4$	C, H			77	III
25	H	Br	H	H	H	181		$C_8H_5BrO_4$		177–178[7]		78	
26	H	H	Br	H	H	168–169 (H_2O)		$C_8H_5BrO_4$		170.5[7]		52	
27	Me	H	Ph	H	H	60–61 (MeOH)	170–180 (1.0)	$C_{16}H_{14}O_4$		62–63[8]	140–160 (0.001)[8]	42	
28	Et	H	Me	H	H		134 (0.3)	$C_{13}H_{16}O_4$	C, H			91	I
29	Me	Me	H	Me	H		114–120 (0.25)	$C_{12}H_{14}O_4$		54–55[24]		25	
30	Me	Me	H	H	Me	78 (MeOH)	178 (0.5)	$C_{12}H_{14}O_4$	C, H			34	III
31	H	Me	H	H	Me	141–142[b]		$C_{10}H_{10}O_4$	C, H	145[26]		75	
32	Me	H	Me	Me	H	53–54 (MeOH–pet. ether)		$C_{12}H_{14}O_4$	C, H			78	III
33a	Me	H	*o*-Phenylene		H	49–51 (MeOH)		$C_{14}H_{12}O_4$		48–50[27]		85	I
33b	Et	H	*o*-Phenylene		H	55 (EtOH)		$C_{16}H_{16}O_4$	C, H			68	I
34	Me	OMe	H	Me	H	84–85 (MeOH–pet. ether)	154 (0.7)	$C_{12}H_{14}O_5$		84.5–85.5[12]		57	II
35	Me	OMe	H	Et	H	89 (MeOH)	210–218 (0.5)	$C_{13}H_{16}O_5$	C, H			25	II
36	H	OMe	H	Et	H	175 (H_2O)		$C_{11}H_{12}O_5$	C, H			88	
37	Et	*o*-Phenylene		H	H	85–88 (acetone)		$C_{16}H_{16}O_4$	C, H	88.5–89.5[28]		71	
38	Me	1,2-Cyclohexylene		H	H	34–35 (MeOH)	134 (0.2)	$C_{14}H_{16}O_4$	C, H		170 (7.0)[29]	84	III
39	Me	OMe	H	H	Me	57–60 (MeOH)	144–148 (0.1)	$C_{12}H_{14}O_5$		58–59[12]		76	II
40	Me	1,2-Cyclopentylene		H	H		130–140 (0.2)	$C_{13}H_{14}O_4$		45[30]	168–172 (4.0)[30]	32	III

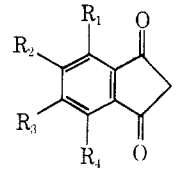
[a] See Experimental Section. [b] Resolidifies to remelt as the anhydride at 144–145°.

Table II. Intermediate Phthalic Anhydrides



Compd	R_1	R_2	R_3	R_4	Mp, °C	Formula	Lit. mp, °C	Yield, %
41	Br	H	H	H	129–130	$C_8H_3BrO_3$	130–131[7]	60
42	H	Br	H	H	104–106	$C_8H_3BrO_3$	104–106[7]	88
43	Me	H	H	Me	144–145	$C_{10}H_8O_3$	143[31]	94
44	OMe	H	H	Me	186–170 (Ac_2O)	$C_{10}H_8O_4$	185–186[12]	85

Table III. Physical Properties of Substituted Indan-1,3-diones



Compd	R_1	R_2	R_3	R_4	Method of preparation[a]	Mp, °C (benzene)	Formula	Analyses	Lit. mp, °C	Yield, %
45	H	H	H	H	A	129			132[14]	64
46	OMe	H	H	H	A	145–147 dec	$C_{10}H_8O_3$	C, H	172–173[17]	86
47	OEt	H	H	H	A	111	$C_{11}H_{10}O_3$	C, H	116–117[32]	41
48	OPr-*n*	H	H	H	A	94–95	$C_{12}H_{12}O_3$	C, H		38
49	OPr-*i*	H	H	H	A	69–70	$C_{12}H_{12}O_3$	C, H	68–70[32]	51
50	OBu-*n*	H	H	H	A	66	$C_{13}H_{14}O_3$	C, H		45
51	OBu-*i*	H	H	H	A	65	$C_{13}H_{14}O_3$	C, H		58
52	Ph	H	H	H	A	125–128	$C_{15}H_{10}O_2$	C, H		80
53	Me	H	H	H	A	125–128	$C_{10}H_8O_2$	C, H	126–128[33]	70
54	Et	H	H	H	A	148	$C_{11}H_{10}O_2$	C,[e] H		66
55	F	H	H	H	C[b]	117–118	$C_9H_5FO_2$	C, H		55
56	Br	H	H	H	C[b]	Decomp >120	$C_9H_5BrO_2$		158–159 dec[34]	66
57	H	Br	H	H	C	152–153[c]	$C_9H_5BrO_2$	C, H, Br	157–160[16]	86
58	H	Ph	H	H	A	116	$C_{15}H_{10}O_2$	C, H		20
59	H	Me	H	H	A	114–116	$C_{10}H_8O_2$	C, H		44
60	Me	H	Me	H	A	137–138	$C_{11}H_{10}O_2$	C, H		28
61	Me	H	H	Me	B	187–188	$C_{11}H_{10}O_2$	C, H		84
62	H	Me	Me	H	A	159	$C_{11}H_{10}O_2$	C, H		48
63	H	*o*-Phenylene		H	A	136 dec	$C_{13}H_8O_2 \cdot H_2O$	C,[d] H		65
64	OMe	H	Me	H	A	172–173	$C_{11}H_{10}O_3$	C, H		77
65	OMe	H	Et	H	A	118	$C_{12}H_{12}O_3$	C, H		60
66	*o*-Phenylene		H	H	A	178 dec	$C_{13}H_8O_2$	C, H		71
67	1,2-Cyclohexylene		H	H	A	97–99	$C_{13}H_{12}O_2$	C, H		37
68	1,2-Cyclopentylene		H	H	A	159–162 dec	$C_{12}H_{10}O_2$	C, H		26
69	OMe	H	H	Me	B	172	$C_{11}H_{10}O_3$	C, H		91
70	Cl	Cl	Cl	Cl	C[d]	Dec	$C_9H_2Cl_4O_2$	C, H, Cl		62

[a] A, Claisen; B, Perkin; C, Knoevenagel. [b] Used *tert*-butyl acetoacetate. [c] Recrystallized from acetone. [d] Calcd, 72.89; found, 73.80. [e] Calcd, 75.85; found, 76.65.

methods of synthesis are described below.

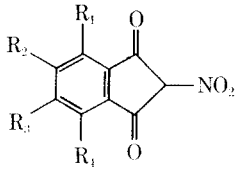
Method A. Claisen Condensation. 5,6-Dimethylindan-1,3-dione (62). A solution of dimethyl 4,5-dimethylphthalate (14.6 g, 0.066 mol) in EtOAc (20 ml) was added to a 50% dispersion of NaH in mineral oil (4.63 g, 0.096 mol of NaH) and the mixture was refluxed at 100° for 4 hr. After the solution was cooled, the yellow solid was filtered and washed well with EtOH-Et_2O (1:1). Treatment of this solid with a hot (80°) solution of concentrated HCl (20 ml) in water (200 ml) over 7 min gave the title compound as a buff solid. After drying *in vacuo* over P_2O_5 and recrystallization from PhH the product, 5.5 g (48%), had mp 159°. *Anal.* ($C_{11}H_{10}O_2$) C, H.

Method B. Perkin Reaction. (a) 3,6-Dimethylphthalidylideneacetic Acid. A mixture of 3,6-dimethylphthalic anhydride (15.3 g, 0.087 mol), freshly fused KOAc (13.3 g), and Ac_2O (27 ml) was heated at 100° for 1 hr and then at 150–155° for a further 4 hr. After the solution was cooled, water (80 ml) was added and the brown solid filtered off and washed well with water and cold methanol. Extraction of the residue with 5% aqueous $NaHCO_3$ followed by acidification of the extract afforded the phthalidylideneacetic acid as a yellow solid. Recrystallization from dioxane gave 7.73 g (41%) of compound melting at 264–265°. *Anal.* ($C_{12}H_{10}O_4$) C, H.

(b) 4,7-Dimethylindan-1,3-dione (61). Sodium methoxide (from Na (8.05 g) in MeOH (80 ml)) was added with vigorous stirring to a solution of 3,6-dimethylphthalidylideneacetic acid (7.5 g, 0.034 mol) in MeOH (200 ml) and the resulting gel was allowed to stand for 2 hr. The red suspension was refluxed at 100° for 5 hr, cooled, and filtered. Addition of the solid to hot (80°) 5 *N* HCl (120 ml) caused spontaneous decarboxylation and generation of the dione. The solid was filtered, dried *in vacuo* over P_2O_5, and recrystallized from PhH to give 5.02 g (84%), mp 187–188°. *Anal.* ($C_{11}H_{10}O_2$) C, H.

Method C. Knoevenagel Reaction. 4-Fluoroindan-1,3-dione (55). 3-Flurorophthalic anhydride (17.8 g, 0.1 mol) was dissolved in Ac_2O (55 ml) containing Et_3N (30 ml) and the solution was treated with *tert*-butyl acetoacetate (17.4 g, 0.11 mol). After standing overnight a mixture of ice (40 g) and concentrated HCl

Table IV. 2-Nitroindan-1,3-diones



Compd	R_1	R_2	R_3	R_4	Mp, °C[a]	Lit. mp, °C	Formula	Analyses	Yield, %	Activity in rat PCA test: ED_{50},[b] mg/kg sc at T_{max}[c]	Activity in rat PCA test: T_{max},[c] min
1	Disodium cromoglycate									6.7 (4.9–9.3, 147, 56)	10
2	H	H	H	H	113–114	113[35]	$C_9H_5NO_4 \cdot 2H_2O$	C, H, N	74	7.4 (5.1–10.6, 49, 84)	0
71	OMe	H	H	H	132–134		$C_{10}H_7NO_5$	C, H, N	79	63 (23–287, 40, 36)	30
72	OEt	H	H	H	95–96		$C_{11}H_9NO_5 \cdot 2H_2O$	C, H, N	46	12 (2.7–105, 42, 30)	30
73	OPr-*n*	H	H	H	114–115		$C_{12}H_{11}NO_5$	C, H, N	27[d]	15 (*e*, –, 18)	0
74	OPr-*i*	H	H	H	80–81		$C_{12}H_{11}NO_5$	C, H, N	59	45 (26–163, 100, 36)	0
75	OBu-*n*	H	H	H	85		$C_{13}H_{13}NO_5$	C, H, N	74	5.7 (4.8–6.7, 51, 24)	20
76	OBu-*i*	H	H	H	75–77		$C_{13}H_{13}NO_5$	C, H, N	77	6.6 (2.1–19.2, 83, 24)	20
77	Ph	H	H	H	119		$C_{15}H_9NO_4$	C, H, N	75	>100	
78	Me	H	H	H	108–109	104–105[36]	$C_{10}H_7NO_4 \cdot 0.5H_2O$	C, H, N	75	47 (33–67, 76, 18)	60
79	Et	H	H	H	98–100		$C_{11}H_9NO_4$	C, H, N	10[f]	>100	
80	F	H	H	H	124		$C_9H_4FNO_4$	C, H, N	84	7.4 (3.4–16.3, 44, 24)	0
81	Br	H	H	H	127–128		$C_9H_4BrNO_4$	C, H, Br,[g] N	51	6.8 (4.2–12, 69, 30)	20
82	H	Br	H	H	127–129	84[17,h]	$C_9H_4BrNO_4$	C, H, Br, N	83	3 (*e*, –, 18)	10
83	H	Ph	H	H	119		$C_{15}H_9NO_4 \cdot 0.5H_2O$	C, H, N	82	19 (*e*, -, 18)	20
84	H	Me	H	H	115–117		$C_{10}H_7NO_4$	C, H, N	36	6.2 (3.8–10.1, 49, 42)	0
85	Me	H	Me	H	111–112		$C_{11}H_9NO_4$	C, H, N	73	12 (*e*, -, 36)	20
86	Me	H	H	Me	108–110		$C_{11}H_9NO_4$	C, H, N	73	16 (*e*, –, 24)	20
87	H	Me	Me	H	113–114		$C_{11}H_9NO_4$	C, H, N	85	0.17 (0.08–0.40, 60, 108)	10
88	H	1,2-Phenylene		H	163–164		$C_{13}H_7NO_4$	C, H, N	41	1.1 (0.43–3.12, 44, 30)	0
89	OMe	H	Me	H	156–157		$C_{11}H_9NO_5$	C, H, N	79	8.3 (*e*, –, 24)	20
90	OMe	H	Et	H	116		$C_{12}H_{11}NO_5$	C, H, N	59	2.5 (0.9–11, 53, 24)	20
91	1,2-Phenylene		H	H	134.5–135.5		$C_{13}H_7NO_4$	C, H, N	29	>20	
92	1,2-Cyclohexylene		H	H	108–109		$C_{13}H_{11}NO_4$	C, H, N	13	>100	
93	Cyclopentylene		H	H	128–130		$C_{12}H_9NO_4 \cdot 0.5H_2O$	C, H, N	30[i]	>100	
94	OMe	H	H	Me	143–146		$C_{11}H_9NO_5$	C, H, N	75	40 (*e*, –, 24)	30
95	Cl	Cl	Cl	Cl	184.5		$C_9HCl_4NO_4 \cdot 3H_2O$	C, H, N	83	>100	

[a] Solvent of recrystallization, water–hydrochloric acid. [b] Figures in parentheses are (95% confidence limits; slope of inhibition/log dose line; number of animals used). [c] T_{max} is the time between sc administration of the drug and challenge to give maximum activity. [d] Nitration <5°. [e] Insufficient data for calculation of confidence limits. [f] Crude starting material. [g] Calcd 29.58; found, 28.65. [h] Dihydrate. [i] 90% unreclaimed starting material.

(36 ml) was added to the dark solution whereby the mixture became warm and decarboxylation proceeded. Addition of a further 150 ml of 5 *N* HCl followed by warming to 75° completed the decarboxylation. Extraction of the acid solution with $CHCl_3$ followed by drying ($MgSO_4$) and evaporation afforded crude fluoroindan-1,3-dione. Recrystallization from benzene gave 9.6 g (55%) of a yellowish solid, mp 117–118°. *Anal.* ($C_9H_5FO_2$) C, H.

2-Nitroindan-1,3-diones. Without exception the indandiones have been nitrated as ethereal suspensions at *ca.* 5° with occasional completion of the reaction over 1 hr at ambient temperature. For those derivatives which crystallized during the reaction, work-up consisted of filtration, washing with 5 *N* HCl, and immediate recrystallization from water–HCl. For compounds which remained in solution, addition of 5 *N* HCl followed by total solvent removal under reduced pressure (<40°) usually afforded a dark oil which crystallized on scratching.

Table IV lists the yields, physical properties, and biological activity of the 2-nitroindan-1,3-diones synthesized, the general procedure being illustrated by the following example.

4-Methoxy-2-nitroindan-1,3-dione (71). To a stirred suspension of 4-methoxyindan-1,3-dione (0.53 g, 0.003 mol) in dry Et_2O (3 ml) cooled in an ice bath was added fuming HNO_3 (*d* 1.52, 1.0 ml) dropwise. After 30 min an aliquot of the thick yellow solid dissolved completely in water, indicating completion of the reaction. Filtration gave a bright yellow solid which after washing well with 5 *N* HCl was recrystallized from water to which *ca.* one-third of its volume of concentrated HCl was added to give, after drying *in vacuo* over P_2O_5–NaOH, 0.53 g (79%) of material, mp 132–134°. *Anal.* ($C_{10}H_7NO_5$) C, H, N.

Rat PCA Test. The PCA test was carried out by a method similar to that previously described.[5,6] Serum containing heat-labile homocytotropic antibody was raised in rats to ovalbumin by a method similar to that described by Mota.[25]

One-tenth milliliter of each of six twofold serial dilutions of the serum in 0.9% saline was injected intradermally into separate sites on the shaved dorsal surface of 250–350-g male Wistar rats; 72 hr later the animals were challenged by iv injection of 0.3 ml of 1% solution of ovalbumin in isotonic saline buffered with pH 7.2 Sorenson buffer mixed with 0.2 ml of a 5% solution of Pontamine Sky Blue in saline. The rats were killed after 20 min and the diameter of the blue wheals at the antibody injection sites were measured. The starting dilution of the serum was adjusted so that there was no response, after challenge, at the site of injection of the highest dilution and a maximum response at the lowest dilution. Typically, six twofold serial dilutions of the serum from $\frac{1}{4}$ to $\frac{1}{128}$ were used.

Compounds were tested for their ability to reduce the diameter of the wheals at the injection sites of dilutions of antibody which on the controls gave less than maximum response.

Each dose of the compound was administered to a group of six animals at a measured time prior to intravenous challenge with ovalbumin. Control groups of six animals were administered the same volume of carrier fluid at the same time prior to challenge. T_{max} was the time between sc administration of a compound and iv challenge at which the compound showed maximum activity. It was determined by injecting sc into rats the approximate ED_{50} of the compound, when given just prior to iv challenge, and challenging the rats in groups of six after 0, 10, 20, 30, 60 and 120 min. The line of best fit for the log dose–response curve and the 95% confidence limits were calculated as described in ref 37.

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