FLUORENE DERIVATIVES AS RADIOPROTECTANT DRUGS

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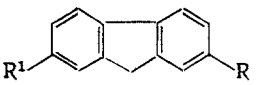
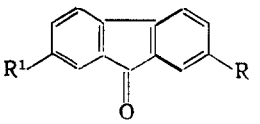
Radioprotectant drugs are being sought among both the classical, proven series of protectants (aminoalkylthiols, isothiuronium salts, thiazolidines, and aminoalkyl thiosulfates), and new types of chemical compounds [8]. There have been no reports in the literature of the promise shown by fluorenes as potential radioprotectants. We here report the results of an examination of the toxic and radioprotectant properties of 15 fluorene derivatives (I-XV), six of which (V, VII, IX, X, XIV, and XV) are new compounds.



I—III, VI—XII



IV, V, XIII—XV

R = H (I, IV), NH_2 (II), CH_2Ph (III), SO_3K (V), $COCH_2Cl$ (VI), $COCH_2SCH_2COAr$(Ar= 2-fluorenyl)(VII), COPh (VIII—X, XIII—XV), COC_6H_4Cl-3 (XI), COC_6H_4OMe-4 (XII); R^1 = H (I—VIII, XI—XIII), SO_3K (IX, XIV), SO_3H (X, XV).

The sulfonic acids (V), (IX), (X), (XIV), and (XV) were obtained by sulfonating the appropriate substituted fluorenes with 65% oleum. The structures of the resulting 2- or 7-sulfonic acids and their potassium salts were confirmed by their elemental analyses and IR spectra. The sulfonic acids (X) and (XV), and the acid obtained from the salt (V) showed absorption at 815-875 cm^{-1} in addition to that for fluorene, in agreement with extraplanar deformational vibrations of two adjacent unsubstituted hydrogen atoms in the aromatic ring (2- or 7-substituted fluorenes), together with absorption at 1050-1110 (SO_2), 3450-3500 (OH), and 1650-1710 cm^{-1} (C=O). Literature reports [9, 10] also show that sulfonation of fluorene gives the 2- or 2,7-sulfonic acids only. The purity of the sulfonation products was established by TLC (alumina, benzene, R_f = 0 for the sulfonic acids and their salts, or 0.39-0.77 for the starting materials).

The thioether (VII) was obtained from 2-chloroacetylfluorene, its structure being confirmed by its elemental analysis and mass spectra. The mass spectrum contained m/z 165, 193, 207, and 446, the first three corresponding to fragments of (VII).

Tests showed that some of these fluorenes substantially increased the radioresistance of animals.

EXPERIMENTAL (CHEMISTRY)

The IR spectra of the compounds were obtained on a Specord-75 spectrometer (East Germany), and mass spectra on an MKh-1303 mass spectrometer (USSR). TLC was carried out on alumina in the systems: a) acetone—acetic acid—concentrated HCl, 1:1:0.15, and b) benzene. The elemental analyses were in agreement with the calculated values.

A commercial sample of fluorene (I), 2-aminofluoroene (II) [11], 2-benzylfluorene (III) [5], fluorenone (IV) [6], 2-chloroacetylfluorene (VI), 2-benzoylfluorene (VIII), 2-(3'-chlorobenzoyl)fluorene (XI), 2-(4'-methoxybenzoyl)fluorene (XII) [1-3, 5], and 2-benzoylfluorenone (XIII) [12] were used in this work.

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Potassium Fluorenone-2-sulfonate (V). A mixture of 0.5 g (3 mmole) of fluorenone and 0.64 g (4.3 mmole) of 65% oleum was stirred at 90°C for 6 h, then treated with 20 ml of water and heated to the boil. The mixture was filtered hot, and the filtrate treated with 0.37 g of potassium carbonate until alkaline. The precipitate was filtered off, washed with water, and recrystallized from water to give 0.68 g (83%) of (V). $C_{13}H_7SO_4K$. R_f 0.52 in system a).

Bis(2-fluorenoylmethyl) Sulfide (VII) was prepared by adding 0.3 g (1.1 mmole) of 2-chloroacetylfluorene in 40 ml of absolute alcohol to a solution of KOEt [from 0.45 g (11 mmole) of potassium in 10 ml of absolute alcohol], saturated with dry H_2S. The mixture was heated at 75°C for 1.5 h, then cooled, and water added. The solid was filtered off and washed with water to give 3.86 g (70%) of (VII) as bright yellow crystals, mp 181-181.5°C (from benzene). R_f 0.3 in system b). $C_{30}H_{22}SO_2$, mol. wt. 446 (mass spectrometry).

Potassium 2-Benzoylfluorene-7-sulfonate (IX) was obtained from (VIII) as described for (V), from 2 g (7 mmole) of 2-benzoylfluorene, 1.7 g (11 mmole) of 65% oleum (reaction time 30 min, temperature 70°C), 30 ml of water, and 3 g of potassium carbonate. Yield 2.6 g (90%). $C_{20}H_{13}SO_4K$. R_f 0.65 in system a).

2-Benzoylfluorene-7-sulfonic Acid (X) was obtained from (IX) by dissolving it in hot, dilute HCl (1:1). The mixture was filtered hot and the crystals of the acid washed a few times with ice-water, mp 279-285°C. R_f 0.65 in system a).

Potassium 2-Benzoylfluorenone-7-sulfonate (XIV) was obtained as for (V). Yield of (XIV) 0.39 g (54%). Increasing the amount of oleum to two mmoles raised the yield to 74%. The same salt was obtained by oxidizing (IX) with sodium dichromate in glacial acetic acid, as described in [12]. $C_{20}H_{11}SO_5K$. R_f 0.48 in system a).

2-Benzoylfluorenone-7-sulfonic Acid (XV) was obtained from (XIV) as described for (X), mp 255-256°C, R_f 0.48 in system a).

EXPERIMENTAL (BIOLOGY)

Tests were carried out on 890 mice of both sexes, strain C57Bl/6, aged 3-3.5 months, weighing 18-20 g. The compounds were administered intraperitoneally in a volume of 0.2 ml of liquid per mouse. Compounds sparingly soluble in water were suspended in a mixture of Tween and water, 1:19 by volume.

Acute toxicities were measured as the LD_{50} values (the dose of the compound causing the deaths of 50% of the animals). The toxicity of some of the compounds was not measured in view of their low solubility (and consequently their low biological availability), or their low toxicity; in these cases the testing limit was the amount of compound which gave a satisfactory suspension in the volume given above.

The animals were irradiated on an IGUR-1 cesium gamma-installation in a single dose of 1.2 cGy/sec. The radiation dose causing the deaths of 95-99% of the mice over 30 days ($LD_{95-99/30}$) was 810 cGy for males, and 855 cGy for females. The compounds were administered at various times before irradiation in amounts of 1/3 of the LD_{50}, or in amounts which, according to prior tests, were absorbed from the peritoneal cavity within one week. Anti-radiation activity was assessed by the 30-day survival rates of the mice, and the mean life-spans of the animals which died (MLA). Radioprotectant activity was examined in respect of the dose of drug, the radiation dose, and the time of administration of the drug to the mice. Also examined were changes in body weight and deaths of the animals, the dose change factor (DCF), and the protectant factor (PF) [7].

Table 1 shows the toxic and radioprotectant properties of the test compounds. Ten of the 15 compounds were insoluble in water. The relatively low toxicities of the compounds, including those soluble in water, is noteworthy. The lowest toxicities (400 and 340 mg/kg) were shown by compounds containing an amino-group and chlorine (II, VI).

The symptoms shown on acute intoxication in the mice at the LD_{50} doses included low mobility, ataxia, abdominal respiration, muscular fibrillation, and paralysis of the rear extremities. Toxic symptoms appeared immediately following administration of the compounds (within a few minutes), subsequently increasing (over a few hours), and remaining present for up to two days. The animals died after 1-5 days.

TABLE 1. Toxic and Radioprotectant Properties of Fluorene Derivatives

Compound	Solvent	Toxicity, LD_{50}, mg/kg	Radioprotectant properties			
			dose given,	time of treatment, h	survival, %	MLA, days
I	Tween–water (1:9)	Over 2000*	500	0,25	13,3±9	11,8
II	The same	400	130	0,25	6,7±6	8,8
II	» »	400	130	3	53,3±13**	16,9
III	» »	1500	500	0,25	13,3±9	7,8
IV	» »	>2000*	500	0,25	53,3±13**	12,3
IV	» »	>2000*	500	3	46,7±13**	14,5
V	Water	800	270	0,25	6,7±6	11,1
VI	Tween–water (1:9)	340	110	0,25	30,0±11**	15,0
VI	The same	340	110	3	33,3±13**	11,3
VII	» »	>2000*	700	0,25	0	6,2
VIII	» »	>2000*	500	0,25	26,0±10**	16,0
VIII	» »	>2000*	500	3	58,1±7**	12,6
IX	Water	940	310	0,25	13,3±9	13,5
IX	»	940	310	3	33,3±13**	10,4
X	»	600	200	0,25	6,7±6	12,7
XI	Tween–water (1:9)	>2000*	500	0,25	35,0±11**	12,7
XI	The same	>2000*	500	3	40,0±11**	16,5
XII	» »	>2000*	500	0,25	45,0±11**	14,5
XIII	» »	>2000*	700	0,25	0	9,3
XIII	» »	>2000*	700	3	33,3±13**	10,0
XIV	Water	620	200	0,25	33,3±13*	13,2
XV	»	1200	400	0,25	46,7±13**	13,8
Control		—	—	0,25—3	4,5±2	10,4

*Maximum dose which could be given by syringe.
**Difference from control significant, $P \leq 0.05$.

TABLE 2. Postradiation Survival of Mice with Respect to Dose of Compound

Compound	Time of treatment	Dose given, mg/kg	Survival, %	MLA, days
II	3	130	53,3±13*	16,9
II	3	50	10±8	10,8
IV	0,25	500	53,3±13*	12,3
IV	0,25	400	40±13*	14,3
IV	0,25	300	20±11	14,5
VIII	3	500	58,1±7*	12,6
VIII	3	400	20±11	11,0
VIII	3	300	0	8,7
Control			5±3	10,8

*Difference from control significant, $P \leq 0.05$.

As will be seen from Table 1, radioprotectant activity was present in ten of the compounds, seven of these increasing the survival of the mice by 30-50%, and three by more than 50%. These levels of activity were observed when the compounds were administered 15 min-3 h before irradiation.

Examination of the relationship of radioprotectant activity to chemical structure showed that antiradiation activity was present in fluorenes with substituents in the 2-position (amino, chloroacetyl, benzoyl, chlorobenzoyl, and methoxybenzoyl) and (or) in the 9-position (carbonyl), and in some of their sulfonated derivatives. Sulfonation in itself increased the biological availability of the compounds (the LD_{50} values decreased from over 2000 to

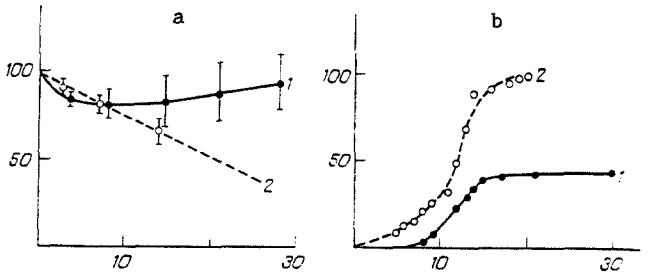

Fig. 1. Changes in body weight (a) and death (b) in protected, irradiated mice. Horizontal axis: days after irradiation. Vertical axis: a) body weight, %; b) deaths, %. (1) 2-Benzoylfluorene (VIII); (2) control .

600-1200 mg/kg), but no significant change in postradiation survival was observed. In the case of (V) and (X), sulfonation resulted in loss of antiradiation activity.

Those compounds showing enhanced radioprotectant properties were subjected to further examination in order to establish the optimum conditions for their use. The results are shown in Table 2 and Fig. 1. As the results show, the optimum radioprotectant properties are shown by 2-aminofluorene in a dose of 130 mg/kg administered three hours before irradiation, by fluorenone in a dose of 400-500 mg/kg administered 0.25-3 h before irradiation, and by 2-benzoylfluorene in a dose of 500 mg/kg three hours before irradiation. The greatest radioprotectant effects were shown by 2-benzoylfluorene, which increased the survival of irradiated mice to 58%, as compared to 95% deaths in the control. 2-Benzoylfluorene increased the radioresistance of the mice over the whole range of lethal doses of radiation, an effect equal to that of the control being obtained at a radiation dose of 135-220 cGy greater than in the control, DCF = 1.27-1.29. The lethal doses of radiation in protected mice following treatment with (VIII) under the optimum conditions were: $LD_{16/30}$ = 635, $LD_{50/30}$ = 785 (761-810), and $LD_{84/30}$ = 970 cGy; for unprotected mice the corresponding doses were 500, 610 (588-632), and 750 cGy.

The effects of the compound were apparent during the period of intestinal and bone marrow death in the animals. In protected mice, the body weight increased considerably after 15 days. The protectant index was greater than 6.

The activity of the fluorenes appears to be due to radiation chemical processes, namely complexation with metals, and the formation of bonds with DNA [13, 14].

A new class of chemical compounds, the fluorenes, have thus been examined for antiradiation activity, and the search has been justified. The results obtained show that further research is desirable, since more than 60% of the compounds tested increased the radioresistance of mice at radiation doses causing the deaths of 95% of the animals in the controls. These protectants are active when given before irradiation for a considerable time. The radioprotectant effect does not exceed 50-60%, but increasing the biological availability, preparing water- and fat-soluble compounds, could result both in increased activity and a reduction in the effective dose.

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