DERIVATIVES OF BENZO(*c*)FLUORENES. III. SYNTHESIS AND BIOLOGICAL ACTION OF SOME DIBASIC DERIVATIVES OF 7-OXO-7*H*-BENZO(*c*)FLUORENE*

Iva VANČUROVÁ, Jiří KŘEPELKA and František ŠMEJKAL

Research Institute for Pharmacy and Biochemistry, 130 60 Prague 3

Received May 26th, 1981

Alkylation of phenols *IIa*—*IIc* by the action of ω-(N,N-dialkylamino)alkyl chlorides *IIIa*—*IIId* in an anhydrous or a two-phase medium (toluene–aqueous potassium hydroxide) gave rise to the dibasic derivatives *IV*—*XI*. In the two-phase medium, alkylation of *IIa* with *IIIc* produced the basic ether *XII* as the main product, a decarboxylation product, and compound *VII*. In biological test compound *IV* showed the strongest antibacterial effects on four kinds of bacteria, was efficacious *in vivo* against the viruses of encephalomyocarditis and vaccinia, and induced the formamation of interferon to the same extent as Tiloron. The antineoplastic effects of the compounds were weaker than those observed with compound *I* (Benfluron) administered to animals with experimental, transplantable tumours.

In the previous communications[1,2] we described derivatives of benzo(*c*)fluorene with various substituents attached to positions 5, 6 and 7, and the results of preliminary tests of biological activity. These showed[2–4] that the derivatives with an ω-(N,N-dialkylamino)alkyl substituent bound *via* oxygen to position 5 were effectual antineoplastic substances so long as an oxo group was bound to position 7. The compound *I* (Benfluron) has been selected out of this group of derivatives for further research, and is passing the stage of the preclinical studies.

With a view to expanding the present-day knowledge of the effect of substitutions on the benzo(*c*)fluorene skeleton upon the biological activity, we now describe a group of disubstituted 7-oxo-7*H*-benzo(*c*)fluorene derivatives, with one basic ω-(N,N-dialkylamino)alkyl residue attached to the 5-position by an ethereal bond, and another attached to the 6-position by an ester bond (compounds *IV*—*XI*, Table I).

The synthesis consisted in alkylation of 5-hydroxy-6-carboxy-7-oxo-7*H*-benzo(*c*)-fluorene and its 3,9-dialkyl analogues *IIa*—*IIc* (ref.[1]) by the action of ω-(N,N-dialkylamino)alkyl chlorides *IIIa*—*IIId*, as previously described[2]; the alkylation was conducted in an anhydrous medium (method *A*) or a two-phase medium (method *B*). In method *A* the compounds *IIIa*—*IIId* were used in the form of bases, in method *B*

* Part LXXX in the series Substances with Antineoplastic Activity; Part LXXIX: This Journal *47*, 1856 (1982).

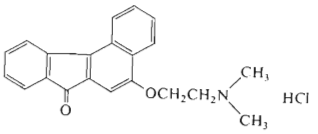
TABLE I

5,6-Disubstituted derivatives of 7-oxo-7*H*-benzo(*c*)fluorene

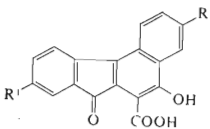
Compound	R^1 / R^2	Formula (mol.mass)	M.p., °C (solvent)	Calculated/Found			
				% C	% H	% N	% Cl
IV[a]	H / $CH_2CH_2N(CH_3)_2$	$C_{26}H_{32}Cl_2N_2O_5$ (523·4)	235—237 (ethanol)	59·65 59·63	6·16 5·95	5·35 5·34	13·54 13·47
V[b]	H / $CH_2CH_2N(C_2H_5)_2$	$C_{38}H_{44}N_2O_{12}$ (720·7)	146—147 (ethanol)	63·32 63·02	6·15 6·22	3·88 3·96	— —
VI	H / $CH_2CH_2N(C_2H_5)_2$	$C_{30}H_{38}Cl_2N_2O_4$ (561·6)	210—212 (ethanol)	64·16 63·99	6·82 6·77	4·99 5·19	12·63 12·46
VII	H / $CH_2CH_2CH_2N(CH_3)_2$	$C_{28}H_{34}Cl_2N_2O_4$ (533·5)	259—261 (ethanol–tetrachloromethane)	63·03 62·74	6·43 6·38	5·25 5·00	13·29 13·58
VIII[c]	H / $CH_2C(CH_3)_2CH_2N(CH_3)_2$	$C_{32}H_{40}N_2O_4$ (516·7)	135—136 (benzene)	74·38 74·58	7·80 7·61	5·42 5·21	— —
IX	H / $CH_2C(CH_3)_2CH_2N(CH_3)_2$	$C_{32}H_{42}Cl_2N_2O_4$ (589·6)	245—247 (ethanol–ether)	65·18 65·43	7·18 7·21	4·75 4·65	12·02 11·89
X	CH_3 / $CH_2CH_2N(C_2H_5)_2$	$C_{32}H_{42}Cl_2N_2O_4$ (589·6)	224—225 (ethanol–benzene)	65·18 64·81	7·18 7·25	4·75 4·64	12·02 12·23
XI	C_2H_5 / $CH_2CH_2N(C_2H_5)_2$	$C_{34}H_{46}Cl_2N_2O_4$ (617·7)	233—235 (ethanol–chloroform)	66·12 66·07	7·50 7·56	4·54 4·81	11·48 11·13

[a] Monohydrate; [b] bishydrogen maleate; [c] base.

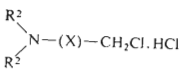
in the form of hydrochlorides. The course of the reaction consisted in the generation of dianions of compounds *IIa*–*IIc* by the action of sodium methoxide or potassium hydroxide, followed by their alkylation, with the formation of an ester or ether bond.



I



IIa: $R^1 = H$
IIb: $R^1 = CH_3$
IIc: $R^1 = C_2H_5$

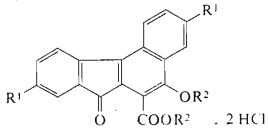


IIIa: $X = CH_2$, $R^2 = CH_3$
IIIb: $X = CH_2$, $R^2 = C_2H_5$
IIIc: $X = CH_2CH_2$, $R^2 = CH_3$
IIId: $X = CH_2C(CH_3)_2$, $R^2 = CH_3$

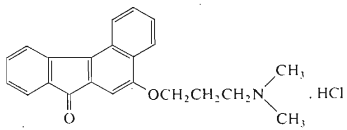
Method *A* proved to give better yields, since in method *B* parts of the starting compounds were decarboxylated by the elevated temperature and the aqueous alkaline medium. The extent of decarboxylation was greatest in the synthesis of compound *VII*, formed concurrently with compound *XII*, whose m.p. and IR spectrum were identical with those of an authentic sample[2]. Alkylation of *IIa* by the action of *IIIa* was possible under the conditions of method *A* only. The alkylation products were characterized in the form of readily crystallizing hydrochlorides, with the exception of compound *VIII*, which crystallized in the form of its base.

The structures of selected compounds were confirmed by IR and ^1H NMR spectra. The presence of a carbonyl group in position 7 was demonstrated by the band in the IR spectrum at 1 710–1 720 cm^{-1}, which is characteristic of conjugated, five-membered ketones. The simultaneous presence of the band at 1 730–1 740 cm^{-1} demonstrated a carbonyl group in an ester bond. With the decarboxylation product of compound *XII* the IR spectrum did not contain this characteristic band.

The compounds *IV*–*XI* were subjected to information tests for antibacterial, antineoplastic, antiviral and interferonogenic activities. In the antibacterial tests *in vitro*, the widest spectrum of activity was observed with the compound *IV* against *Streptococcus pyogenes* CI, *Staphylococcus pyogenes aureus*, *Streptococcus faecalis* and *Escherichia coli*, with the minimum inhibitory concentration being 1·56–6·25 μg/ml, depending on the species. The compounds exhibited no action against yeast



IV–XI



XII

and fungi. In the screening assays for antineoplastic effects in animals with experimental tumours (S 180, HK, Sa 37, Kr 2 and Y) we observed reduction of size of tumours or extended survival of the animals, depending on the doses and the mode of administration. None of the compounds investigated matched compound *I* in antineoplastic activity. Some details on efficacy of the compounds and methodology of evaluation are described elsewhere[3,4]. In assessment of antiviral efficacy of the compounds in mice, as against that of Tiloron[5] as standard, the most efficacious against the viruses of vaccinia and encephalomyocarditis proved to be compound *IV*, which was as effectual as Tiloron. in both *p.o.* and *s.c.* administration. Comparable results were also obtained with compounds *V* and *XI*, but in *s.c.* application only. In tests for interferogenic activity in *p.o.* administration against encephalomyocarditis virus, best results were obtained with compound *IV*. In studying the kinetics of formation of interferon in blood serum of mice, maximum levels on L-cells were found after 24 h; the course of the level in relation to time was the same as that with Tiloron. More details on the antiviral assays and the kinetics of the formation of interferon will be described elsewhere[6].

EXPERIMENTAL

The melting points were determined on the Kofler block and are not corrected. Samples for elemental analyses were dried over phosphorus pentoxide at a pressure of 70 Pa and temperatures proportional to their melting points. Homogeneity of the samples and composition of the reaction mixtures were followed by TLC on reflex foils Silufol UV_{254} (Kavalier), monitored by quenching of UV light of 254 nm. The reaction mixtures were fractioned on a column of Kieselgel 60 reinst (Merck), employing usually a 30-fold weight of the sample. ^{1}H NMR spectra were measured with an apparatus Tesla BS 487 C (80 MHz); 10% solutions in deuteriochloroform or hexadeuteriodimethyl sulphoxide, and tetramethylsilane as internal standard were used. IR spectra (in KBr pellets) were read on a spectometer Perkin–Elmer 577. Mass spectra were measured with an apparatus MS-9.

Alkylation of Compounds *IIa–IIc* by the Action of ω-(N,N-Dialkylamino)alkyl Chlorides *IIIa–IIId*

Method A: To a solution of sodium methoxide (2 to 3 mol equivalents) in methanol was added chlorobenzene and compound *IIa* (1 mol equivalent). The mixture was boiled and methanol was distilled off (bath temperature 130°C). Then a solution of base *IIIa–IIId* (2–3 mol equi-

valents) in chlorobenzene[2] was added to the boiling mixture, which was kept refluxing for 5 to 8 h. After cooling down it was repeatedly extracted with 20% aqueous potassium hydroxide (half volume) until the blue colouration had disappeared, then with an equal volume of water and a saturated solution of sodium chloride. The organic layer was dried with anhydrous magnesium sulphate and concentrated. The residue was purified by column chromatography (*VII*), crystallization (*VIII*), or was used directly to prepare the dihydrochloride (*IV*, *VI*, *VII*, *IX*) or bis-hydrogen maleate (*V*). The addition salts were prepared by dissolving the base (distillation residue) in ethanol and acidification with an ethanolic solution of hydrogen chloride to pH 3 (in the case of the dihydrochlorides) or with 2·2 mol equivalents of maleic acid (compound *V*).

Method B: To a mixture of toluene and water (in a volume ratio of 2·5 : 1 to 5 : 1) was added potassium hydroxide (6 mol equivalents), compound *IIa*–*IIc* (1 mol equivalent) and hydrochloride of *IIIb* or *IIIc* (2·2 mol equivalents), and the reaction mixture was boiled under a reflux condenser for 5 to 15 h. The toluene layer was then separated and worked up as in method *A*.

IV: Method *A*, *IIa* (0·58 g, 0·002 mol), sodium (0·09 g, 0·004 mol), 2 ml of methanol, 5 ml of chlorobenzene, base *IIIa* (0·43 g, 0·004 mol) in 5 ml of chlorobenzene, reflux for 8 h. Yield 0·3 g (29%) after crystallization. IR spectrum: 1 610, 1 590 (Ar), 1 712 (C=O, conjugated ketone), 1 740 cm^{-1} (C=O, ester). ^1H NMR spectrum (hexadeuteriodimethyl sulphoxide): δ 7·20–8·80 (m, 8 H, ArH), 4·90 (bt, 2 H, OCH_2), 4·51 (bt, 2 H, OCH_2), 2·85 (s, 6 H, NCH_3), 2·85 (s, 6 H, NCH_3).

V: Method *A*, *IIa* (17·4 g, 0·06 mol), sodium (2·7 g, 0·12 mol), 45 ml of methanol, 150 ml of chlorobenzene, base *IIIb* (16·3 g, 0·12 mol) in 100 ml chlorobenzene reflux for 6 h. The residue of the base (22·0 g) was dissolved in 50 ml of ethanol and 15·34 g (0·13 mol) of maleic acid was added. The mixture was heated to the boiling temperature and allowed to cool down and crystallize. Yield 28·4 g (66%).

VI: Method *B*, *IIa* (5·8 g, 0·02 mol), *IIIb* (7·5 g, 0·044 mol), potassium hydroxide (6·7 g, 0·12 mol), 40 ml of water, 200 ml of toluene, reflux for 5 h. Yield 4·8 g (43%). IR spectrum: 720, 778 (1,2-disubstituted Ar), 1 580, 1 603, 1 620 (Ar), 1 710 (C=O), conjugated ketone, 1 730 (C=O, ester, 2 400, 2 480, 2 580 cm^{-1} (NH^+).

VII: Method *A*, *IIa* (4·36 g, 0·015 mol), sodium (0·69 g, 0·03 mol), 15 ml of methanol, 30 ml of chlorobenzene,base *IIIc* (3·65 g, 0·03 mol) in 30 ml of chlorobenzene, reflux for 4h. Yield 6·7 g (84%). IR spectrum: 715, 775 (1,2-disubstituted Ar), 1 570, 1 600, 1 612 (Ar), 1 715 (C=O, conjugated ketone), 1 730 (C=O, ester), 2 480, 2 590, 2 660 cm^{-1} (NH^+).

VII + *XII*: Method *B*, *IIa* (0·58 g, 0·002 mol), *IIIc* (0·70 g, 0·0044 mol), potassium hydroxide (6·7 g, 0·012 mol), 4 ml of water, 20 ml of toluene, reflux for 10 h. The crude residue was purified by column chromatography with benzene as eluant. The base in the first fractions was converted into its hydrochloride. Yield 0·42 g (39%) of compound *XII*, m.p. 242–243°C (ethanol), IR spectrum 1 585, 1 610 (Ar), 1 715 (C=O, conjugated ketone), 2 490, 2 610 cm^{-1} (NH^+); *cf.* ref.[2]. The next fractions analogously gave 0·2 g (18·7%) of compound *VII*.

VIII: Method *A*, *IIa* (0·58 g, 0·002 mol), sodium (0·09 g, 0·004 mol), 2 ml of methanol, 7 ml of chlorobenzene, base *IIId* (0·6 g, 0·004 mol) in 5 ml of chlorobenzene, reflux for 6 h. The crude residue was purified by crystallization. Yield 0·38 g (36·8%). ^1H NMR spectrum (deuteriochloroform): δ 7·00–8·60 (m, 8 H, ArH), 4·25 (s, 2 H, OCH_2), 3·92 (s, 2 H, OCH_2), 2·35 (s, 2 H, NCH_2), 2·21 (s, 2 H, NCH_2), 2·28 (s, 12 H, NCH_3), 1·11 (s, 6 H, CCH_3), 1·00 (s, 6 H, CCH_3).

IX: 0·26 g (0·0005 mol) of base *VIII* in 3 ml of ethanol, the solution was adjusted to pH 3 with ethanolic hydrogen chloride and cooled down. Yield 0·18 g (61%). IR spectrum: 1 590, 1 610 (Ar), 1 712 (C=O, conjugated ketone), 1 740 cm^{-1} (C=O, ester).

X: Method *B*, *IIb* (4·14 g, 0·013 mol), *IIIb* (5·34 g, 0·028 mol), potassium hydroxide (4·36 g, 0·078 mol), 40 ml of water, 150 ml of toluene, reflux for 15 h. Yield 4·45 g (53·0%). IR spectrum: 838 (trisubstituted Ar), 1 585, 1 620 (Ar), 1 715 (C=O, conjugated ketone), 1 735 (C=O, ester), 2 500, 2 600 cm^{-1} (NH^+). Mass spectrum: *m/e* 516 (M^+, $C_{32}H_{40}N_2O_4$).

XI: Method *B*, *IIc* (3·34 g, 0·01 mol), *IIIb* (3·78 g, 0·022 mol), potassium hydroxide (3·36 g, 0·06 mol), 20 ml of water, 50 ml of toluene, reflux for 8 h. Yield 3·7 g (60%). Mass spectrum: *m/e* 545 (M^+, $C_{34}H_{44}N_2O_4$). IR spectrum: 835 (trisubstituted Ar), 1 550, 1 615 (Ar), 1 710 (C=O, conjugated ketone), 1 735 (C=O, ester), 2 460 cm^{-1} (NH^+).

The elemental analyses were performed by Mrs J. Komancová (Analytical Department, head Dr J. Körbl), the antibacterial tests by Mrs M. Pivoňková (Microbiological Department, head Dr J. Holá), and the antineoplastic tests by Mrs S. Pokorná (Pharmacological Department, head Dr J. Grimová). Acknowledgement is also due to the firm BASF Aktiengesselschaft, Ludwigshafen, F.R.G., for a gift of 3-(N,N-*dimethylamino*)-2,2-*dimethylpropan*-1-*ol*.

REFERENCES

1. Křepelka J., Roubík J., Holubek J., Vančurová I.: This Journal *47*, 1258 (1982).
2. Křepelka J., Vančurová I., Holubek J., Mělka M., Řežábek K.: This Journal *47*, 1856 (1982).
3. Mělka M., Křepelka J., Vančurová I., Řežábek K.: Arzneim.-Forsch., in press.
4. Pujman V., Černochová S.: Neoplasma *28*, 715 (1981).
5. Andrews E. R., Fleming R. W., Grisar J. M., Kihm J. C., Wenstrup D. L., Mayer G. D.: J. Med. Chem. *17*, 882 (1974).
6. Šmejkal F., Křepelka J.: Acta Virol., in press.

Translated by J. Salák.