SOME ANALOGS OF 4-(2′,4′-DIFLUOROBIPHENYL-4-YL)-2-METHYL-4-OXOBUTANOIC ACID: SYNTHESIS AND ANTIINFLAMMATORY ACTIVITY

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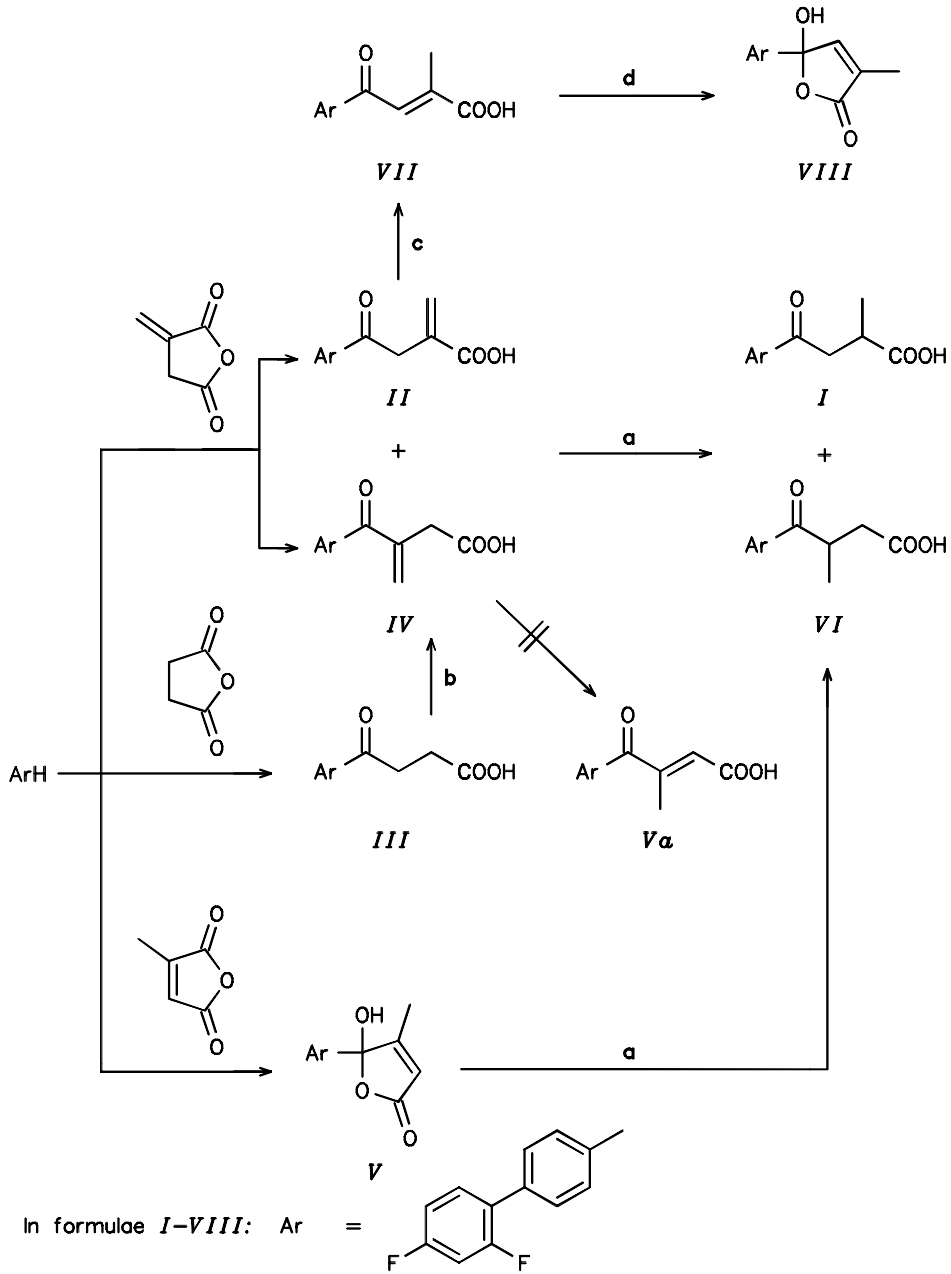
Analogs of 4-(2′,4′-difluorobiphenyl-4-yl)-2-methyl-4-oxobutanoic acid (*I*, flobufen), containing a double bond (*II*, *IV*, *V*, *VII*, *VIII*) or a methyl group in position 3 (*VI*) were prepared. Their antiinflammatory activity was evaluated and compared with that of flobufen. None of the mentioned analogs reached the activity of the standard. Isomerization of the unsaturated derivatives is connected with a shift of the double bond, *Z–E* transformation or lactonization. Reaction conditions and spectra of the compounds prepared are described.

In connection with the development of antiinflammatory arylalkanoic acids we synthesized a series of derivatives with the connecting chain between the carboxyl and the aromatic nuclei modified by the presence of carbonyl. We used[2,3] QSAR analysis for the optimization of the structure of these aryloxoalkanoic acids. Simultaneously, we studied the prolongation of the antiinflammatory effect using suitable structural changes based on a plausible hypothesis[4] of biotransformation. Discovery of 4-(2′,4′-difluorobiphenyl-4-yl)-2-methyl-4-oxobutanoic acid (*I*) with a high and protracted antiinflammatory effect[5,6] and low gastrotoxicity was the result of our investigation. This compound is now in the third phase of clinical trials under the name of flobufen. Inhibition of leukotriene (LT) biosynthesis as well as LTB_4 antagonism are important for the mechanism of its antiinflammatory effect[7].

EXPERIMENTAL

Melting points were determined on a Kofler block. ^{1}H NMR spectra were measured in a 5% solution in hexadeuteriodimethyl sulfoxide with trimethylsilylpropionic acid as internal standard on a 487s-80 MHz Tesla NMR spectrometer. ^{13}C NMR spectra were measured in a 15% solution under the same conditions. ^{1}H-COSY and NOESY spectra were measured in a 12.5% solution in hexadeuteriodimethyl sulfoxide with trimethylsilylpropionic acid at 60 °C on a DPX 250 (Bruker) NMR spectrometer. The ^{13}C NMR spectra of compounds *I*, *II*, *IV–VIII* are given in Table I. IR spectra (cm^{-1}) were measured both in a 3% solution in chloroform or in a KBr pellet on a Shimadzu (Japan) IR-435 spectrometer. The purity of compounds *I–VIII*, the presence of 3-methylene isomer *IV*



a) 0.5 MPa H_2, Pd/C, 20 °C, dioxane; b) pyridine/piperidine, $(CH_2O)_n$, 60 °C, 10 h; c) $(C_2H_5)_3N$, 20 °C, 8 h, H_2O; d) sunlight, 20 °C, 2 days, $(C_2H_5)O$

SCHEME 1

in compound *II*, and the course of isomerization of compound *II* to compound *VII* were determined by HPLC on a 3.9 × 300 mm μ-Bondapak C_{18} column (Waters) in an acetonitrile–phosphate buffer, pH 3.5 (50 : 50, v/v) using a liquid chromatograph consisting of an LC-6A (Shimadzu) pump, a Rheodyne 7125 injector and an SPD-6AV (Shimadzu) detector. TLC evaluation of compounds *I*–*VIII* was performed on silica gel (FP KG F_{254}, Merck) as stationary phase and benzene–chloroform–acetic acid (30 : 20 : 2.5, v/v) as mobile phase with twofold development. The following R_F values were observed: 0.44 (*I*), 0.42 (*II*), 0.35 (*IV*), 0.25 (*V*), 0.40 (*VI*), 0.40 (*VII*), and 0.26 (*VIII*), respectively. The presence of the 3-methyl isomer *VI* in compound *I* was evaluated by GLC on a Fractovap 2450 chromatograph (Carlo Erba) on a fused silica capillary column (25 m, i.d. 0.22 mm) coated with SE-54 (thickness 0.2 mm). Prior to the measurements, the compounds were converted into methyl esters by treatment with diazomethane. The purity of citraconic anhydride was determined by HPLC on a 3.9 × 300 mm μ-Bondapak CN column (Waters) using a liquid chromatograph Water (U.S.A.) consisting of an 8000 A pump, U6K injector and M 440 detector, with dichloromethane–heptane–acetic acid (40 : 60 : 1, v/v) as mobile phase. Retention times: citraconic anhydride 4.3, itaconic anhydride 5.2, citraconic acid 8.8 min.

4-(2′,4′-Difluorobiphenyl-4-yl)-2-methyl-4-oxobutanoic Acid (*I*)

Compound *I* was prepared by hydrogenation of 2-methylene derivative *II*; the reaction conditions were mentioned elsewhere[8,9]. ^{1}H NMR spectrum: 8.05 d, 2 H, *J* = 8.0 (H-3); 7.76 bd, 2 H, *J* = 8.0 (H-2); 7.10–7.70 m, 3 H (H-3′, H-5′, H-6′); 2.70–3.60 m, 3 H (CH_2CH); 1.10 d, 3 H, *J* = 7.0 (CH_3). IR spectrum (KBr): 1 712 (COOH), 1 678 (CO), 1 615, 1 605 (aromatic).

4-(2′,4′-Difluorobiphenyl-4-yl)-2-methylene-4-oxobutanoic Acid (*II*)

The title compound was prepared by the Friedel–Crafts reaction of itaconic anhydride with 2,4-difluorobiphenyl; the reaction conditions were described elsewhere[8,9]. The crude substance

TABLE I
^{13}C NMR spectra of the aliphatic moiety of compounds *I*, *II*, *IV* – *VIII*

Compound	^{13}C NMR signals				
	C-4	C-3	C-2	C-1	CH_3
I	197.76	41.34	34.43	176.43	16.70
II	196.75	41.68	138.93	167.62	128.03[a]
IV	196.15	141.47	37.79	172.02	128.70[a]
V	106.93	168.62	116.86	170.80	13.97
VI	202.35	36.75	37.27	173.00	17.29
VII	191.82	130.94	141.25	168.51	14.64
VIII	[b]	136.25	128.81[c]	171.46	12.06

[a] Signal of the $=CH_2$ group; [b] signal is overlapped; [c] identification is uncertain.

containing about 2% (HPLC) of the 3-methylene isomer *IV* was purified by crystallization from methanol–water (4 : 1, v/v) and acid *II* (the content of *IV* lesser than 0.1%) with m.p. 174–176 °C in the yield of 67% was obtained. For $C_{17}H_{12}F_2O_3$ (302.3) calculated: 67.55% C, 4.00% H, 12.57% F; found: 67.46% C, 4.52% H, 12.50% F. ^{1}H NMR spectrum: 8.12 d, 2 H, *J* = 9.5 (H-3); 7.70 bd, 2 H, *J* = 9.5 (H-2); 7.10–7.60 m, 3 H (H-3′, H-5′, H-6′); 6.26 d, 5.50 d, 2 H, *J* = 2.0 (CH_2=); 4.10 s, 2 H (CH_2). IR spectrum (KBr): 1 692 (COOH), 1 683 (CO).

4-(2′,4′-Difluorobiphenyl-4-yl)-4-oxobutanoic Acid (*III*)

Aluminum chloride (13.2 g, 0.1 mol) was gradually added at 20 °C to a mixture of 2,4-difluorobiphenyl (19.0 g, 0.1 mol) and of succinic anhydride (9.0, 0.09 mol) in 1,2-dichloroethane (75 ml). After stirring for 3 h at 20 °C, the mixture was poured on ice (200 g) and hydrochloric acid (75 ml). The precipitate was extracted with ether (twice 200 ml). After washing with water the ether solution was extracted with 5% NaOH (twice 50 ml). The alkaline solution was washed with ether, filtered with charcoal and acidified with 50% H_2SO_4 (15 ml). The precipitated crude substance was filtered, washed with water and, after crystallization from 70% acetic acid (60 ml), compound *III* (10.5 g, 40%) with m.p. 131–132 °C was obtained. For $C_{16}H_{12}F_2O_3$ (290.3) calculated: 66.20% C, 4.17% H, 13.90% F; found: 66.42% C, 4.14%H, 13.30% F.

4-(2′,4′-Difluorobiphenyl-4-yl)-3-methylene-4-oxobutanoic Acid (*IV*)

Piperidine (1.0 g) was added at 60 °C to a mixture of acid *III* (7.25 g, 0.025 mol) and paraformaldehyde (1.2 g, 0.04 mol) in 15 ml of pyridine and the mixture was stirred for 10 h at 60 °C. The solution was cooled to 10 °C, filtered and poured into a mixture of ice (85 g) and hydrochloric acid (25 ml). The precipitate was filtered off and washed with water. Crystallization from 60% methanol (100 ml) afforded compound *IV* (4.8 g, 64%) with m.p. 144–148 °C. For $C_{17}H_{12}F_2O_3$ (302.3) calculated: 67.55% C, 4.00% H, 12.57% F; found: 67.27% C, 4.02% H, 12.43% F. ^{1}H NMR spectrum: 7.88 d, 2 H, *J* = 8.0 (H-3); 7.70 bd, 2 H, *J* = 8.0 (H-2); 7.20–7.60 m, 3 H (H-3′, H-5′, H-6′); 5.75 s, 6.14 s, 2 H (CH_2=); 3.50 s, 2 H (CH_2). IR spectrum (KBr): 1 708 (COOH), 1 647 (CO).

Lactonol of (*Z*)-4-(2′,4′-Difluorobiphenyl-4-yl)-3-methyl-4-oxobut-2-enoic Acid (*V*)

Aluminum chloride (26.0 g, 0.195 mol) was gradually added at 10 °C to a mixture of 2,4-difluorobiphenyl (19.0 g, 0.1 mol) and citraconic anhydride* (11.2 g, 0.1 mol) in dichloromethane (80 ml). The mixture was stirred 4 h at 10 °C and 2 h at 20 °C and then poured into a mixture of ice (200 g) and hydrochloric acid (65 ml). The organic phase was separated, washed with water, the solvent evaporated and the residue crystallized from toluene. Compound *V* (17.7 g, 58.7%) with m.p. 181–182 °C was used for the preparation of acid *VI*. For biological testing, the substance was once more crystallized from toluene (in a 74% yield) and acid *V* with m.p. 185–187 °C was obtained. For $C_{17}H_{12}F_2O_3$ (302.3) calculated: 67.55% C, 4.00% H, 12.57% F; found: 67.61% C, 4.09% H, 12.36% F. ^{1}H NMR spectrum: 7.00–7.80 m, 7 H (H-arom.); 6.12 bq, 1 H (CH=); 1.90 bs, 3 H (CH_3). IR spectrum (KBr, Nujol): 1 685 (COOH), 1 672 (CO); ($CHCl_3$): 1 758 (lactone).

* Prepared by 4-h heating of a mixture of citraconic acid (22.0) and acetic anhydride (47.3 ml) at 70 °C. The following distillation afforded citraconic anhydride with b.p. 95 – 98 °C/2.4 kPa and 100% purity (HPLC) in a 59.0% yield.

4-(2′,4′-Difluorobiphenyl-4-yl)-3-methyl-4-oxobutanoic Acid (*VI*)

Compound *V* (30.2 g, 0.1 mol) was hydrogenated on Pd/C catalyst (2.0 g, Hereus K 0.224, 3% of Pd in dry material) in dioxane (100 ml) at 20 °C and 0.5 MPa in an autoclave. After the hydrogenation was finished (the equivalent of hydrogen was consumed), the catalyst was filtered off and the filtrate was concentrated under diminished pressure (0.4 kPa). The residue was stirred with acetone (50 ml), filtered and crystallization from toluene afforded compound *VI* (9.6 g, 31.6%) with m.p. 151–153 °C. For $C_{17}H_{14}F_2O_3$ (304.3) calculated: 67.10% C, 4.64% H, 12.49% F; found: 66.85% C, 4.70% H, 12.60% F. ^{1}H NMR spectrum: 8.05 d, 2 H, J = 8.0 (H-3); 7.04–7.80 m, 5 H (other H-arom.), 3.92 m, 1 H (CH); 2.70 m, 2 H (CH_2); 1.12 d, 3 H, J = 7.0 (CH_3). IR spectrum (KBr): 1 700 (COOH), 1 678 (CO).

(*E*)-4-(2′,4′-Difluorobiphenyl-4-yl-2-methyl-4-oxobut-2-enoic Acid (*VII*)

Triethylamine (25.0 g) was added to a stirred suspension of acid *II* (12.1 g, 0.04 mol) in water (70 ml) at 20 °C. The originating yellow solution was stirred at 20 °C for 8 h, then poured into ice (150 g) and acidified with hydrochloric acid (1 : 1, v/v, 45 ml) to pH 2. The precipitate was filtered, washed with water and crystallized from 2-propanol after drying. Acid *VII* with m.p. 198 199.5 °C was obtained in an amount of 8.7 g (72%). For $C_{17}H_{12}F_2O_3$ (302.3) calculated: 67.55% C, 4.00% H, 12.57% F; found: 67.33% C, 4.08% H, 12.50% F. ^{1}H NMR spectrum: 8.05 bd, 2 H (H-3); 7.20–7.80 m, 6 H (other H-arom., CH=); 2.10 d, 3 H, J = 2.0 (CH_3). IR spectrum (KBr): 1 694 (COOH), 1 662 (CO).

Lactonol of (*Z*)-4-(2′,4′-Difluorobiphenyl-4-yl)-2-methyl-4-oxobut-2-enoic Acid (*VIII*)

Acid *VII* (3.02 g, 0.01 mol) was dissolved in ether (250 ml) and the yellow solution was exposed at 20 °C to sunlight until the yellow color disappeared (2 days). The clear, almost colorless, solution was evaporated under reduced pressure (0.4 kPa) and the residue (one spot according to TLC with R_F lower than the starting *VII*) was twice crystallized from toluene. Lactonol *VIII* (1.8 g, 59.6%) with m.p. 153–154.5 °C and of purity higher than 99% (HPLC) was obtained. For $C_{17}H_{12}F_2O_3$ (302.3) calculated: 67.55% C, 4.00% H, 12.57% F; found: 67.82% C, 4.07% H, 12.42% F. ^{1}H NMR spectrum: 6.96–7.87 m, 8 H (H-arom., CH=); 3.37 bs, 1 H (OH); 1.94 d, 3 H, J = 1.17 (CH_3). IR spectrum ($CHCl_3$, KBr, Nujol): 1 781 (lactone).

Biological Assays

Inhibition of carrageenan edema was evaluated by the method of Winter[10]; the experimental conditions are described elsewhere[11]. Inhibition of experimental pleuritis was evaluated by the method of Hidaka[12] in a group of Wistar Han female rats pretreated with 0.5% of carrageenan in saline by intrapleural injection. The tested compounds, in suspension with gum arabic, were applied orally in a single dose 1 h before the application of carrageenan. The volume of exudate from the pleural cavity, the number and content of cells (determined by a Sysmex counter) were compared with those of untreated animals. Arachidonic acid-induced ear inflammation in mice was performed by the method of Opas[13], the ear-lobe inflammation was induced by application of 20 µl arachidonic acid solution in acetone. The compound was given orally 16 h before edema induction. The degree of ear-lobe hyperemia and the weight of ear lobes were evaluated 1 h after application of arachidonic acid. The results were expressed as percent inhibition in comparison with untreated control.

Inhibition of LTB_4 biosynthesis: the production of LTB_4 was determined in rat polymorphonuclear cells from pleural exudate elicited by heat-inactivated rat serum[14]. The cells were stimulated by the $Ca^{(2+)}$ ionophore A23187 (Sigma) and incubated with various concentrations of tested drugs. LTB_4 was determined in supernatants using commercial RIA kit (Amersham). For the LTB_4 receptor bind-

ing study a slightly modified method of Cheng et al.[15] was used. The membrane fraction was prepared from male guinea-pig spleen; 2 mg of membranes was incubated with 0.3 nM ^{3}H-LTB$_4$ at 25 °C for 30 min in 100 μl incubated mixture. Nonspecific binding was determined in the presence of 0.1 μM LTB$_4$. Membranes were filtered through Whatman GF/C paper, three times washed with buffer; radioactivity was measured by liquid scintillation spectrometry and the specific binding of ^{3}H-LTB$_4$ to the receptor was determined. The biological activities of compounds *I*, *II*, *IV–VIII* are given in Table II.

RESULTS AND DISCUSSION

A by-product of the Friedel–Crafts reaction of 2,4-difluorobiphenyl with itaconic anhydride[8] is the 3-methylene derivative *IV* (Scheme 1). The reaction is highly regioselective; nevertheless, this undesirable by-product yields by hydrogenation 4-(2′,4′-difluorobiphenyl-4-yl)-3-methyl-4-oxobutanoic acid (*VI*), i.e. a regioisomer of flobufen (*I*). These compounds were prepared for a comparative evaluation of the biological effects with flobufen. The 3-methylene analog *IV* was obtained by the reaction of 4-(2′,4′-difluorobiphenyl-4-yl)-4-oxobutanoic acid (*III*) with formaldehyde in the presence of piperidine and pyridine[16]. Its hydrogenation led to compound *VI*, the 3-methyl isomer of flobufen. The shift of the carboxyl band in the IR spectrum of

TABLE II
Biological activities of compounds *I*, *II*, *IV–VIII*[a]

Compound	Mortality[b] %	Inhibition of CE[c], %	Pleuritis[d], %			Ear inflammation[e], %		5-LO inhibition	LTB$_4$ binding
			A	B	C	A	B		
I	50	68	46	61	42	27	46	37.8[f]	37[f]
II	0	57	29	51	27	11	2[g]	33.0[f]	200[f]
IV	40	56	16	1[g]	16	14	5[g]	31.5[f]	–[i]
V	20	62	6[g]	17	8[g]	0	9[g]	79/30[h]	1 000[f]
VI	0	33	6[g]	15[g]	20	4[g]	5[g]	81/30[h]	200[f]
VII	20	65	15	24	18[g]	4[g]	10[g]	56.0[f]	63/100[h]
VIII	–[i]	48	–[i]	–[i]	–[i]	–[i]	–[i]	95/30[h]	1 000[f]

[a] The doses of compounds for the evaluation of antiinflammatory activity were 25 mg/kg; [b] acute toxicity was evaluated after a single dose of 500 mg/kg of the compound to female rats (10 animals), expressed as percent mortality on the 7th day after administration; [c] carrageenan edema; [d] percent inhibition: A volume of exudate, B number of cells, C cellularity in volume unit; [e] percent inhibition: A weight of ear lobes, B degree of ear-lobe hyperemia; [f] inhibition is expressed as IC$_{50}$ in μM; [g] statistically insignificant data; [h] percent inhibition/concentration in μg/ml; [i] not determined.

compound *II* (1 693 cm^{-1}) compared with the same band in compound *IV* (1 708 cm^{-1}) indicates conjugation of the carboxyl group with the double bond. A similar shift was observed for the carbonyl band in compound *IV* (1 683 cm^{-1}), again as a consequence of greater conjugation. ^1H NMR spectra gave different signals for methylenic protons: 4.10 s for $COCH_2$ in *II* and 3.50 s for CH_2COOH in *IV*, and confirm the assumed structures. The reverse shift of $=CH_2$ protons in the same pair of compounds is also important from this point of view.

Lactonol *V* accompanies in a small amount the preparation of unsaturated acid *IV* as a consequence of a shift of the double bond to the α-position. The *Z*-configuration at this bond permits subsequent lactonization. Alternatively, we prepared lactonol *V* using the Friedel–Crafts reaction of 2,4-difluorobiphenyl with citraconic anhydride (Scheme 1). Its structure was confirmed by the IR spectrum in chloroform with a band at 1 758 cm^{-1}, corresponding to the carbonyl group in lactonol. In contrast, the IR spectrum in a KBr pellet or Nujol produced the band at 1 685 cm^{-1}, corresponding to the carbonyl group. ^{13}C NMR spectra also proved the presence of a lactonol moiety, giving only one signal (170.80) corresponding to the C atom in the carbonyl group. With the acyclic form (*IV* as an example) two downfield signals at 196.15 (CO) and 172.02 (COOH) were observed. The ^{13}C NMR signals of aliphatic moieties of compounds *I–VIII* are summarized in Table I. The existence of lactonol *V* was also shown by TLC and HPLC. Attempts to isomerize the 3-methylene derivative *IV* into *Va* of *E*-configuration by the use of an organic base (in analogy to 2-methylene derivative *II*) were unsuccessful.

To synthesize the series of flobufen analogs, we prepared 2-methylbut-2-enoic acid *VII* from 2-methylene derivative *II* by a shift of the double bond in the presence of triethyl amine using a modified method of Lutz[17]. *E*-Configuration of *VII* was proved by long-range interactions (e.g., H-3 to 2-CH_3) using ^1H-COSY 90 and NOESY. Similarly to the derivative of 4-(2′-chlorobiphenyl-4-yl)-2-methylene-4-oxobutanoic acid[18] (Itanoxon), acid *VII* was isomerized in sunlight to the corresponding *Z*-isomer. Its spontaneous cyclization led to lactonol *VIII*, characterized by the IR band at 1 751 cm^{-1}. This structural change was reflected in a change of chromatographic behavior (TLC, HPLC). In the case of lactonol *VIII*, too, no other signal beyond 170 ppm, corresponding to the second carbonyl, was found in the ^{13}C NMR spectrum.

The antiinflammatory activity of the compounds prepared was evaluated in the chosen models of experimental inflammation in vivo, as well as in inhibition of LTB_4 biosynthesis and in binding to LTB_4 receptors in vitro. The results, summarized in Table II, were compared with those of flobufen *I*. It was mentioned that shifting of the methyl group to position 3 in derivative *VI* led to a decrease of toxicity accompanied by a lower antiinflammatory effect. Compound *VI* is the sole accepted impurity in flobufen with a maximum content of 0.3%. Substitution of methyl with methylene in derivative *II* manifests itself by a significant decrease of activity only in the model of ear inflammation. The same decrease was mentioned for other derivatives of flobufen

IV–VIII. The mechanism of this type of inflammation is obviously connected with the pathological effect of leukotrienes in the inflammation process. Derivatives *II–VIII* are active in the inhibition of LTB_4 biosynthesis, similarly as flobufen. A significant difference was found in the binding of these compounds to LTB_4 receptors where flobufen is evidently more active. The possibility exists that the influence of flobufen on the mechanism of this type of inflammation is connected with its LTB_4 antagonism. It can be concluded that the structural changes in derivatives *II–VIII* did not offer any more effective compound than flobufen.

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REFERENCES

1. Kuchar M., Brunova B., Rejholec V., Cepelak V.: Collect. Czech. Chem. Commun. *51*, 2617 (1986).
2. Kuchar M., Maturova E., Brunova B., Grimova J., Tomkova H., Holubek J.: Collect. Czech. Chem. Commun. *53*, 1862 (1988).
3. Kuchar M., Rejholec V., Roubal Z., Maturova E. in: *QSAR in Drug Design and Toxicology* (D. Hadzi and B. Jerman-Blazic, Eds), p. 124. Elsevier, Amsterdam 1987.
4. Chiccarelli F. S., Eisner H. J., vanLear G. E.: Arzneim.-Forsch. *30*, 407 (1980).
5. Grimova J., Vortel V., Lapka R., Lastovicka J.: Cesk. Fysiol. *37*, 249 (1988).
6. Grimova J., Panajotovova V., Lapka R., Lastovicka J.: Cesk. Fysiol. *39*, 133 (1990).
7. Lastovicka J., Panajotovova V., Grimova J.: J. Biopharm. Sci. *1*, 127 (1990).
8. Kuchar M., Poppova M., Zunova H., Knezova E., Vosatka V., Prihoda M.: Collect. Czech. Chem. Commun. *59*, 2705 (1994).
9. Kuchar M., Vosatka V., Sturc A.: Czech. Appl. PV 3413-91 (1991).
10. Winter J.: Proc. Soc. Exp. Biol. Med. *111*, 544 (1962).
11. Grimova J., Kuchar M., Pavlikova L., Nemecek O.: Cesk. Farm. *29*, 305 (1980).
12. Hidaka T.: J. Pharm. Pharmacol. *38*, 242 (1986).
13. Opas E. E., Bonney E. Y., Humes J. L.: J. Invest. Dermatol. *84*, 253 (1985).
14. Palmer R. M. J., Salmon J. A.: Immunology *50*, 65 (1983).
15. Cheng J. B., Cheng E. I.-P., Kohl F., Townley R. G.: J. Pharmacol. Exp. Ther. *236*, 126 (1986).
16. Kampo K., Ogawa K., Takeshita K., Nakaike S., Tomisawa K., Sota K.: Chem. Pharm. Bull. *36*, 2050 (1988).
17. Lutz R. E., Bailey P. S., Dien C. K., Rinker J. W.: J. Am. Chem. Soc. *75*, 5039 (1953).
18. Rieu J.-P., Mouzin G., Cousse H., Boucherle A.: J. Pharm. Sci. *69*, 49 (1980).