Synthesis of Furodiketochroman and bis-Furocoumarin Derivatives and their Biological Activity

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Abstract □ A number of substituted furodiketochroman derivatives (III_{a-f}) have been synthesized by fusion of aromatic aldehydes with 5-hydroxybergapten and 5-hydroxyisopimpinellin. On the other hand, when the reaction was carried out in a solvent, the corresponding bis-furocoumarin derivatives (IV_{c-n}) were obtained. The anticoagulant effect of compounds $III_{a,b,d}$ and $IV_{b,c,f,g,i,k}$, was tested. They failed to demonstrate any significant effect. The effect of the tested compounds on the arterial blood pressure was studied. Compounds IV_c, III_d, III_b, IV_b, IV_g, IV_k and IV_l showed lowering effects on the normal systolic blood pressure of anaesthetized rats in a decreasing manner.

Some furocoumarins are known to possess photodynamic activity as they have the property, when applied to the skin, of provoking erythema followed by pigmentation on successive exposure to sunlight or ultraviolet light. Moreover they possess tuberculostatic[1], molluscidal[2] and antibacterial properties[3,4]. The anticoagulant activity of 3,3-methylene-bis-(4-hydroxycoumarin) was rated as very potent[5]. The minimum structural requirements for activity are an intact 4-hydroxycoumarin residue or a hydrogen atom.

Therefore, it became of interest to synthesize some bis-furocoumarins in order to test their biological activity.

Chemistry

The starting materials 4-methoxy-5-hydroxy-7H-furo(3,2-g)(1)benzopyran-7-one(5-hydroxybergapten) (I_a) and 4,9-dimethoxy-5-hydroxy-7H-furo(3,2-g)(1)benzopyran-7-one(5-hydroxyisopimpinellin) (I_b) were prepared from the naturally occuring furochromones "visnagin and khellin" through alkaline hydrolysis followed by Claisen condensation using ethyl carbonate.
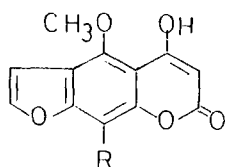
I_a, R = H
I_b, R = OCH_3

When either benzaldehyde or anisaldehyde were fused with I_a or I_b, the 6-(benzal)-(III_{ac}) or 6-(p-methoxybenzal)-5,7-furodiketochroman derivatives ($III_{b,d}$) were obtained. All the compounds showed the presence of two $\supset C = O$ groups at 1680-1695 cm^{-1} and 1710-1735 cm^{-1} (see table I for IR and UV data).
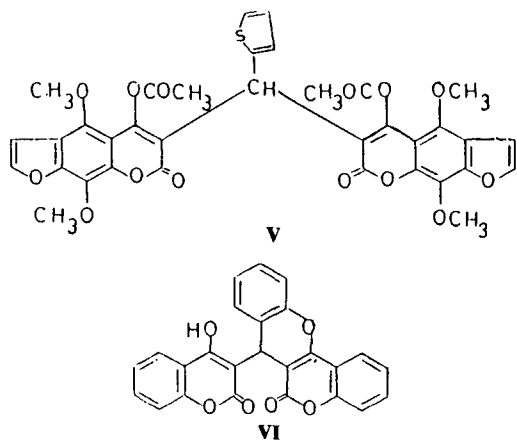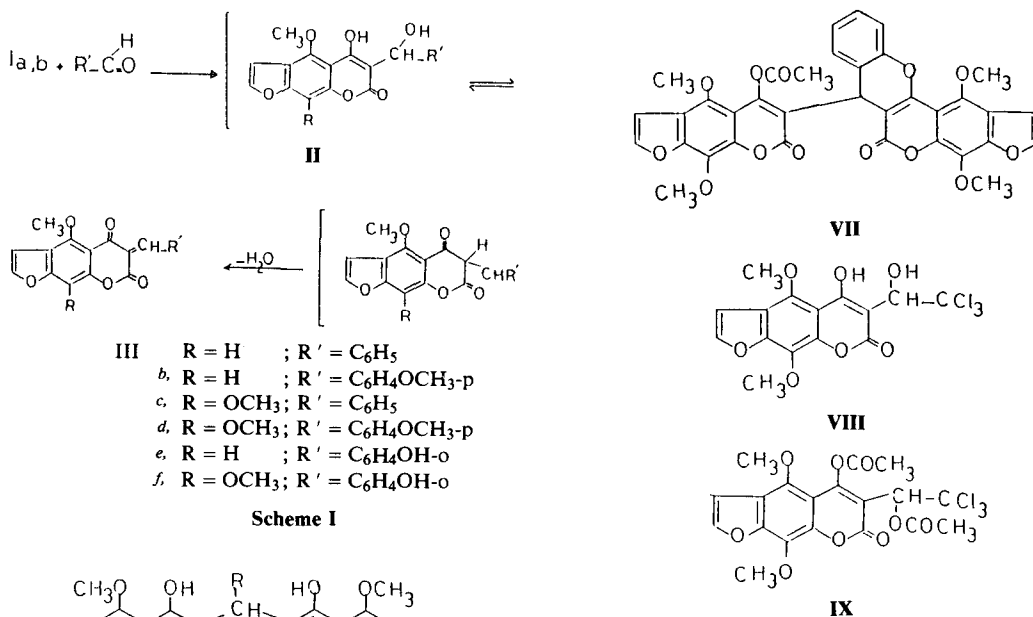
The probable mechanism for the formation of III involves an aldol condensation between the aldehyde and the furocoumarin derivatives (I_a or I_b) to yield the aldol (II) which dehydrates to an α,β-unsaturated ketone (III) as illustrated in scheme I.

On the other hand, the reaction of either I_a or I_b with acetaldehyde, benzaldehyde or anisaldehyde in ethanol-chloroform mixture gave the corresponding dicoumarol analogues (IV $_{c-f}$). This is in agreement with the findings of Schonberg et al.,[6] who treated I_a or I_b with formaldehyde to give the dicoumarol analogues ($IV_{a,b}$)

Fusion of either I_a or I_b with p-bromobenzaldehyde, 3-pyridine aldehyde or 2-thiophene aldehyde gave the corresponding 6,6'-(p-bromobenzylidene)-bis- ($IV_{g,h}$), 6,6'-(3-pyridylidene)-bis ($IV_{i,j}$) or 6,6'-(2-thienylidene)-bis-(5-hydroxybergapten or 5-hydroxyisopimpinellin) (IV_{k-l}).

Compounds IV_{c-n} showed one $\supset C = O$ group at 1700-1735 cm^{-1} which is characteristic of -pyrone ring (see table I for IR and UV data).

When compound IV_l was treated with pyridine and acetic anhydride, no cyclization occured as suggested previously[7], instead the diacetate(V) was ob-

Ia,b + R'–C.O(H) ⟶

II

III

	R = H	; R' = C$_6$H$_5$
b,	R = H	; R' = C$_6$H$_4$OCH$_3$-p
c,	R = OCH$_3$;	R' = C$_6$H$_5$
d,	R = OCH$_3$;	R' = C$_6$H$_4$OCH$_3$-p
e,	R = H	; R' = C$_6$H$_4$OH-o
f,	R = OCH$_3$;	R' = C$_6$H$_4$OH-o

Scheme I

IV

a,	R = H	; R' = H
b,	R = OCH$_3$;	R' = H
c,	R = OCH$_3$;	R' = CH$_3$
d,	R = H	; R' = C$_6$H$_5$
e,	R = OCH$_3$;	R' = C$_6$H$_5$
f,	R = OCH$_3$;	R' = C$_6$H$_4$OCH$_3$-p
g,	R = H	; R' = C$_6$H$_4$Br-p
h,	R = OCH$_3$;	R' = C$_6$H$_4$Br-p
i,	R = H	; R' = C$_5$H$_4$N-m
j,	R = OCH$_3$;	R' = C$_5$H$_4$N-m
k,	R = H	; R' = C$_4$H$_3$S
l,	R = OCH$_3$;	R' = C$_4$H$_3$S
m,	R = H	; R' = C$_6$H$_4$OH-o
n,	R = OCH$_3$;	R' = C$_6$H$_4$OH-o

V

VI

VII

VIII

IX

tained.

However, fusion of I_a or I_b with salicylaldehyde led to two products, a low melting product soluble in ethanol $III_{e,f}$ and an insoluble high melting derivative ($IV_{m,n}$).

It is of interest to report that Link and co-workers[8] found that the reaction of an equimolecular mixture of salicylaldehyde with 4-hydroxycoumarin in alcohol for 10 minutes gave 3-(O-hydroxybenzal)-2,4-diketochroman. While reaction of 4-hydroxycoumarin with salicylaldehyde in a molar ratio (2:1) gave 3-[6-oxo(1)benzopyrano(4,3-b)(1)benzopyran-7-yl]-4-hydroxycoumarin (**VI**)[8].

In order to obtain the cyclized form, VI_n was treated with pyridine and acetic anhydride to give the cyclized acetate VII.

The reaction of 5-hydroxyisopimpinellin with chloral hydrate in ethanol led to the formation of 6-(α-hydroxy-β,β,β-trichloroethyl)-5-hydroxyisopimpinellin (**VIII**) postulated in the mechanism of formation of the 5,7-furodiketochromans (**III**).

This is in agreement with the findings of Ikawa and Link[10] who isolated the corresponding 3-(α-hydroxy-β,β,β-trichloroethyl)-4-hydroxycoumarin with chloral hydrate. Moreover, the compound **VIII** gave the diacetate **IX** upon treatment with acetic anhydride.

The compounds were determined with the results of their physical data and NMR spectra and elemental analysis shown on Table I, II and III.

Table I. The IR and UV data of the synthesized furodi-ketochroman and bis-furocoumarin derivatives

Compound	IR signals in cm^{-1}		UV bands in nm
	$>C=O$	$-OH$	
III_c	1735,1680		238,250,268,315
III_d	1735,1680		230,240,272,320
III_e	1710,1680	3400	215,240,250,325
III_f	1720,1695	3400	
IV_c	1700	3185-3660	
IV_d	1720	3200-3700	
IV_e	1710	3140-3360	
IV_f	1715	3140-3700	
IV_g	1720	3180-3670	
IV_h	1725	3200-3400	
IV_i	1735	3200-3700	
IV_j	1720	3180-3400	
IV_k	1710	3200-3400	
IV_l	1715	3270	
IV_m	1710	3200-3700	
IV_n	1710	3100-3350	
V	1710-1730		240,255,315
VII	1700-1720		
VIII	1730	3220,3380	

Pharmacological Results

Studies on the anticoagulant effects of the tested compounds indicated that all compounds tested failed to demonstrate any significant anticoagulant effect. (Therefore the numberical data are not presented hare.) However the introduction of the furan moiety to the coumarin ring led to the loss of the anticoagulant effect which has been observed previously in the case of dicoumarol derivatives[5].

The effect o the tested compounds on the arterial blood pressure of rats is shown in Table IV. It was observed that some of the tested compounds showed lowering effects on the normal systolic blood pressure of anaesthetised rats in a decreasing manner (VI_c, III_d, III_b, IV_b, IV_g, IV_k and IV_j). Compounds III_a and IV_f failed to demonstrate any effect on the arterial blood pressure in doses up to 1gm/kg body weight.

It was observed that in the furodiketochroman serise ($III_{a,b,d}$) substitution of the phenyl ring in the inactive III_a with a OCH_3 group at the p-position enhanced the activity e.g. III_b and III_d. Moreover, III_d is more active than III_b due to the presence of an extra OCH_3 group.

In the bis furocoumarin series ($IV_{b,c,f,g,i,k}$) it was

found that the replacement of one benzylidene hydrogen in IV_b by a CH_3 ground as in IV_c increased activity. While replacement by an anisyl group aboilishes the activity (IV_f). On the other hand, replacement by a p-bromophenyl (IV_g) or a heterocyclic ring (IV_i or IV_k) diminishes the activity.

EXPERIMENTAL

General Synthetic Procedure:
Formation of 6-substituted-5,7-furodiketochromans III_{a-d}: General Procedure:

A mixture of 1m mole of the furocoumarin (I_a or I_b) and benzaldehyde or anisaldehyde (2m mole) was refluxed for 30 minutes (III_a and III_c) or for 3 hours (III_b and III_d). The products obtained were washed with ether and crystallized from a suitable solvent as brown crystals of III_{a-d}. They gave no colour reaction with aqueous ferric chloride solution.

Formation of 6,6'-substituted methylene bis-(5-hydroxyfurocoumarins IV_{c-l}): General Procedure:

A. Solvent Method

Dissolve 1m mole of the furocoumarin(I_a or I_b) in ethanol-chloroform mixture (10 m/:30 m/) then add 1m mole of the aldehyde. After 8 hours reflux, the solvent was evaporated and the product so obtained was crystallized from the appropriate solvent to give IV_{c-f} as colourless-pale brown crystals.

B. Fusion Method

A mixture of 10m mole of I_a or I_b and the aldehyde (1m mole) was refluxed for 1 hour, then left to cool. The product so obtained was washed with ether, then crystallized from a suitable solvent to give IV_{g-l} as yellow-brown crystals.

Acetylation of IV_l and IV_n: Dissolve 1 gm of IV_l or IV_n in 5 m/ pyridine and 5 m/ acetic anhydride. The reaction mixture was stirred for 12 hours, then cooled and acidified with dilute hydrochloric acid. The solid so formed was filtered off and washed with hot acetic acid to obtain buff crystals of the diacetate V and VII, respectively.

Table II. The NMR and mass data of the synthesized furodiketochroman and bis furocoumarin derivatives

Compound	^{1}H NMR in ppm	Mass Spectra
III_c in DMSO	3.8, 4.5 (s,3H each, OCH_3), 7.2-8.0 (m, 7H,5 arom. Hs + 2 furan Hs); 6.95(broad s,1H, benzal H).	M^+ at m/z 350
III_d		M^+ at m/z 380.06
III_f in $CDCl_3$	3.85, 4.05 (s,3H each, OCH_3), 6.85, 7.5 (d,1H each, C_3 and C_2 furan HS, J = 1.96 Hz), 7.3-7.7 (m,4 arom. Hs), 7.9 (s,1H benzal H at C_6), 9.74 (s,1H,OH).	M^+ at m/z 366
IV_c in $CDCl_3$	1.45 (d,3H,CH_3, J = 6.0 Hz), 3.2 (q, 1H,CH-CH_3, J = 5.5 Hz), 4.17, 4.19, 4.22 (s,3H each, OCH_3), 6.84, 7.00, 7.57, 7.72 (d,1H each, J = 1.96 Hz, C_3 and C_2 furan Hs).	
IV_e in DMSO	3.95, 4.0, 4.05, 4.15 (s,3H each, OCH_3), 4.6 (s,1H, benzyliden H at C_6), 7.15-8.3 (m,9H,5 arom. Hs + 4 furan Hs), 10.05 (s,2H,OH).	
IV_f in $CDCl_3$	3.8, 3.9, 3.95, 4.1, 4.2 (s,3H each, CH_3), 4.6 (s,1H benzylidene at C_6), 6.7-7.8 (m,8H, 4 arom. Hs + 4 furan Hs).	
IV_l in $CDCl_3$	4.1, 4.15, 4.2, 4.25 (s,3H each, OCH_3), 4.7 (s,1H, CH<), 7.0 (d,1H, furan H at C_3, J = 1.96 Hz), 7.6 (d,1H, furan H at C_2, J = 1.96 Hz), 7.2-7.35 (doublet of doublet, 1H, thiophen H at C_4), 7.8-8.25 (m,2H, thiophen Hs at C_3 and C_5).	
IV_n in $CDCl_3$	4.03, 4.1, 4.15, 4.25 (s,3H each, OCH_3), 5.8 (s,1H, – CH< at C_6); 6.85 (d,2H,C_3 furan Hs, J = 1.96 Hz), 7.75 (d,2H,C_2 furan HS, J = 1.96 Hz), 7.0-7.6 (m,4H, arom.Hs), 9.72 (s,3H,OH).	M^+ at m/z 626: (628-H_2)*
V in DMSO	2.7 (s,6H, two $OCOCH_3$), 4.6 (s,1H, – CH<), 3.85, 3.9, 4.0, 4.2 (s,3H each, OCH_3), 6.6-8.8 (m,7H,C_2 and C_3 furan Hs, C_3, C_4 and C_5 thiophen Hs).	
VIII in DMSO	3.9, 4.0 (s,3H each, OCH_3), 5.5 (s,CH in the side chain at C_6), 8.2, 7.25 (d,1H each, furan C_2 and C_3 Hs, J = 1.96 Hz).	
IX in DMSO	2.2, 3.15 (s,3H each, $COCH_3$), 3.75, 3.85 (s,3H each, OCH_3), 6.15 (s,1H,CH at the side chain), 7.3, 8.2 (d,1H each, C_3 and C_2Hs, J = 1.96 Hz).	

* This phenomenon is frequently observed in aromatic compounds[9].

Reaction of I_a or I_b with salicylaldehyde: A mixture of 1m mole of the furocoumarin (I_a or I_b) and 1m mole of salicylaldehyde was refluxed for 30 minutes. The reaction mixture was left to cool, then washed with petroleum ether (b.p 60-80°). The solid so obtained was crystallized from ethanol to give $III_{e,f}$ respectively. While the insoluble $IV_{m,n}$ were crystallized from acetone. III_e, III_f, IV_m and IV_n were obtained as yellow, orange, buff and yellow crystals respectively.

6-(α-hydroxy- β,β,β-trichloroethyl)-5-hydroxyisopimpinellin (VIII) was obtained *via* method A (solvent method) as previously described, as golden yellow crystals.

The acetate IX. Reflux 0.3 g of compound VIII with 3 m*l* of acetic anhydride for 3 hours, then the reaction mixture was worked up as usual.

Anticoagulant effect:

The anticoagulant effects of the tested compounds $III_{a,b,d}$ and $IV_{b,c,f,g,i,k}$ were evaluated using the whole-blood clotting time and the one-stage prothrombin time (Medway)[11].

The tested compounds were dissolved in 50%

Table III.

Compound	m.p.	Solv. of cryst.	Yield (%)	Formula	Calcd. C	Calcd. H	Calcd. N,S or Hal.	Found C	Found H	Found N,S or Hal.
III$_a$	242-3	benzene	65	$C_{19}H_{12}O_5$	71.25	3.75		71.42	4.00	
III$_b$	204	acetone	55	$C_{20}H_{14}O_6$	68.57	4.00		68.42	4.24	
III$_c$	180-2	acetic acid	80	$C_{20}H_{14}O_6$	68.57	4.00		68.34	4.18	
III$_d$	203-4	chloroform	80	$C_{21}H_{16}O_7$	66.32	4.21		66.54	4.44	
III$_e$	192	ethanol	30	$C_{19}H_{12}O_6$	67.86	3.57		67.74	3.81	
III$_f$	160-1	ethanol	26	$C_{20}H_{14}O_7$	65.57	3.83		65.87	4.15	
IV$_c$	182-4	pet.ether(60-80°)	60	$C_{28}H_{22}O_{12}$	61.09	4.00		61.12	4.21	
IV$_d$	261-2	ethanol-Chlorof.	50	$C_{31}H_{20}O_{10}$	67.39	3.62		67.23	3.51	
IV$_e$	223-4	ethanol	45	$C_{33}H_{24}O_{12}$	64.70	3.92		64.56	4.01	
IV$_f$	226	ethanol	40	$C_{34}H_{26}O_{13}$	63.55	4.05		63.46	4.32	
IV$_g$	277-78	chloroform	50	$C_{31}H_{19}O_{10}Br$	58.95	3.01	12.68*	58.78	2.82	13.03*
IV$_h$	252-3	acetic acid	45	$C_{33}H_{23}O_{12}Br$	57.31	3.33	11.58*	57.56	3.52	11.70*
IV$_i$	270	acetic acid	45	$C_{30}H_{19}NO_{10}$	65.10	3.43	2.53**	64.85	3.33	2.78**
IV$_j$	260	acetic acid	40	$C_{32}H_{23}NO_{12}$	62.64	3.75	2.28**	62.40	4.03	2.18**
IV$_k$	258-60	acetone	40	$C_{29}H_{18}O_{10}S$	62.36	3.22	5.58***	62.03	3.17	5.65***
IV$_l$	149-50	ethanol	50	$C_{31}H_{22}O_{12}S$	60.19	3.56	5.18***	60.33	3.71	5.45***
IV$_m$	235	acetone	35	$C_{31}H_{20}O_{11}$	65.49	3.52		65.29	3.87	
IV$_n$	238-40	acetone	45	$C_{33}H_{24}O_{13}$	63.06	3.82		63.27	4.09	
V	280	acetic acid	25	$C_{35}H_{26}O_{14}S$	59.83	3.70	4.56***	60.05	3.39	4.24***
VII	256-7	acetone	27	$C_{35}H_{24}O_{13}$	64.42	3.68		64.18	3.92	
VIII	215	acetone	70	$C_{15}H_{11}O_7Cl_3$	43.96	2.69	26.00****	43.83	2.95	25.97****
IX	170	ethanol	60	$C_{19}H_{15}O_9Cl_3$	46.20	3.04	21.58****	45.91	2.87	21.29****

*Bromine; ** Nitrogen; *** Sulphur; **** Chlorine

Table IV. Effect of the tested compounds on the systolic blood pressure of rats

							Dose g/kg B.Wt. and Decrease %						
III$_b$		III$_d$		IV$_b$		IV$_c$		IV$_g$		IV$_i$		IV$_k$	
0.025	10.15* ±1.3	0.005	18.69 ±0.65	0.010	8.23 ±1.12	0.005	21.3 ±0.88	0.020	7.31 ±1.11	0.025	9.03 ±0.38	0.010	22.52 ±0.29
0.050	15.67 ±0.67	0.010	22.67 ±1.77	0.020	20.1 ±1.3	0.010	39.40 ±1.77	0.025	11.0 ±0.59	0.050	12.5 ±0.75	0.020	11.94 ±0.75
0.100	20.33 ±0.45	0.020	40.34 ±0.88	0.025	24.0 ±0.59	0.020	54.65 ±1.33	0.050	14.67 ±0.33	0.100	38.12 ±1.05	0.025	27.10 ±1.74

*Mean values ± standard error

propylene glycol in distilled water and were given orally to groups of rats (120-150 gm body weight and 5-6 animals in each group) in doses ranging from 0.25 to 2.0 gm/kg body weight. The control group received propylene glycol in distilled water only. Blood samples were taken 6 and 12 hours after treatment and the clotting time and prothrombin time were determined, results were calculated as mean values ± standard error.

Effect on arterial blood pressure in rats:

This was investigated according to the method described by Mcleod *et al.*[12] Rats were anaesthetised using urethane, the trachea, one common carotid artery and the external jagular vein were canulated.

The carotid artery was connected to a pressure transducer, which was connected to a Harvard 2120 biograph recorder. Tested compounds dissolved in 50% propylene glycol in distilled water were injected into the jagular vein and washed in with 0.2 m*l* saline. The blood pressure was determined before and after injection of the tested compounds and the propylene glycol solution. Each dose was tested and the mean% effect was calculated.

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