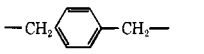
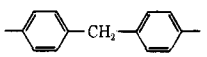
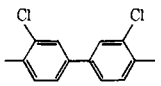
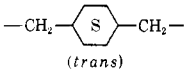
TABLE II

N,N′-BIS(AZIRIDINEACETYL)-α,ω-DIAMINES

Compd	R	Crystn solvent	Mp, °C	Yield, %	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
9		Benzene	188–190	19[a]	61.3	6.61	20.4	61.2	6.60	20.4
10		Benzene	124–125	18	61.3	6.61	20.4	61.1	6.82	20.6
11		Benzene–cyclohexane	128–129	20	49.0	4.70	16.3	48.8	4.41	16.4
12		Benzene–cyclohexane	148–149.5	30	63.6	7.30	18.5	63.3	7.30	18.3
13		Benzene–cyclohexane	115.5–118	15[a]	69.2	6.64	15.4	69.5	6.78	15.6
14		Benzene	224–226	25[a]	57.3	4.81	13.6	57.3	4.75	13.5
15	 (*trans*)	Benzene–cyclohexane	146–148	56[a]	62.3	9.15	18.2	62.2	9.03	18.2

[a] Tetrahydrofuran was used as the solvent for the reaction.

TABLE III

EFFECTS OF COMPOUNDS ON THE REPRODUCTION OF HOUSEFLIES

Compd	Wt % in feed	No. of flies	% egg hatch[a,b] Days of oviposition 1	2	3	4	5	6	7
Control	...	400	94	94	92	96	94	83	—
	...	250	92	97	90	98	88	91	87
9	1	300	3	13	4	31	26	67	23
	1	200	2	2	6	5	6	3	19
10	1	300	1	35	44	32	35	54	39
11	1	300	52	67	57	67	—	—	—
	0.1	300	88	66	35	83	—	—	—
	0.01	300	92	89	85	95	—	—	—
12	1	300	0	5	0	3	/	15	/
	1	200	/	/	/	/	/	/	/
	0.1	200	3	1	/	/	/	/	/
	0.01	200	21	15	17	20	75	17	28
13	1	300	56	33	48	52	70	83	38
14	1	300	91	98	85	—	—	58	88
15	1	300	1	5	/	/	/	/	/
	0.1	300	0	—	/	35	30	36	40
	0.01	300	73	82	88	87	89	71	79

[a] Slant lines indicate no eggs laid. [b] Horizontal lines indicate no eggs set.

had previously been found to possess only minimal activity as a housefly chemosterilant.[1] A comparison between the activities of **9**, **13**, and **14** would indicate that the distance between the alkylating groups of **13** and **14** is too large for maximal activity.

In Japanese quail (*Coturnix coturnix* japonica), **15** at dietary levels not adversely affecting egg production has been found to have a marked effect on reproduction as evidenced by low egg fertility and hatchability. Details of these studies are being reported elsewhere.

Acknowledgment.—This work was supported by U. S. Public Health Service Grant GM-11491. We wish to thank V. Tovar for assistance with the biological studies.

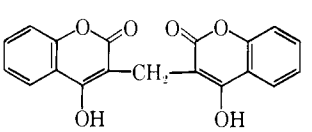
Structure and Anticoagulant Activity of Bridge-Substituted Dicoumarols[1]

R. B. ARORA,[2] N. R. KRISHNASWAMY, T. R. SESHADRI, S. D. S. SETH,[2] AND B. R. SHARMA

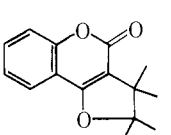
Department of Chemistry, University of Delhi, Delhi 7, and Department of Pharmacology, All India Institute of Medical Sciences, New Delhi-16, India

Received June 7, 1966

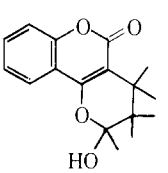
Studies by Overman, *et al.*,[3] and Mentzer, *et al.*,[4] on the anticoagulant activity of coumarin derivatives showed that the minimum structural requirement was a 4-hydroxycoumarin unit with a substituent in position 3 bearing a suitably placed carbonyl group. For maximal activity a bis arrangement as in dicoumarol (I) was considered to be necessary.



I



II



III

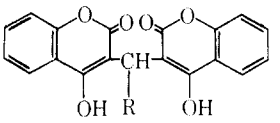
(1) Some of the results presented in this paper are taken from the theses of S. D. S. S. (All India Institute of Medical Sciences) and B. R. S. (Delhi University).

(2) All India Institute of Medical Sciences.

(3) R. S. Overman, M. A. Stahman, C. F. Huebner, W. R. Sullivan, L. Spero, D. G. Doherty, M. Ikawa, L. Graf, S. Roseman, and K. P. Link, *J. Biol. Chem.*, **153**, 5 (1944).

(4) C. Mentzer, P. Meunier, J. Lacocq, O. Billet, and D. Xuong, *Bull. Soc. Chim. France*, **12**, 430 (1945).

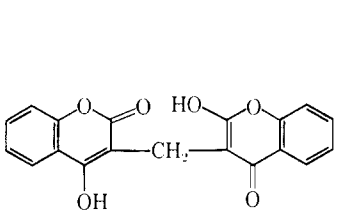
TABLE 1
3,3′-BENZYLIDENEBIS-4-HYDROXYCOUMARINS



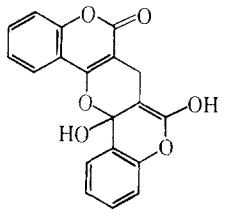
No.	R	Solvent	Mp, °C	Formula	Calcd, % C	Calcd, % H	Found, % C	Found, % H
1	C_6H_5	Dioxane	228–229	$C_{25}H_{16}O_6$				
2	o-ClC_6H_4	EtOH–EtOAc	186–188 dec	$C_{25}H_{15}ClO_6$	67.2	3.4	67.2	3.3
3	p-ClC_6H_4	EtOH–EtOAc	250–252	$C_{25}H_{15}ClO_6$	67.2	3.4	66.5	3.7
4	o-$NO_2C_6H_4$	EtOH–EtOAc	183–184 dec	$C_{25}H_{15}NO_8$	65.6	3.3	65.0	3.6
5	p-$NO_2C_6H_4$	EtOH–EtOAc	233–234	$C_{25}H_{15}NO_8$	65.6	3.3	65.8	3.9
6	m-OHC_6H_4	Dioxane	258–261 dec	$C_{25}H_{18}O_7$	70.1	3.8	70.1	3.8
7	p-OHC_6H_4	EtOH–EtOAc	212–215	$C_{25}H_{18}O_7$	70.1	3.8	70.3	4.5
8	o-$OMeC_6H_4$	Dioxane	208–210	$C_{26}H_{18}O_7$	70.6	4.1	70.4	4.1
9	m-$OMeC_6H_4$	Dioxane	249–250	$C_{26}H_{18}O_7$	70.6	4.1	70.0	4.2
10	p-$OMeC_6H_4$	Ethyl ether	242 dec	$C_{26}H_{18}O_7$				
11	p-$(CH_3)_2NC_6H_4$	a	210 dec	$C_{27}H_{24}O_6N$				
12	$C_6H_4CH{=}CH$	Cyclohexanone–EtOH	232–234	$C_{27}H_{16}O_6$	74.1	3.9	73.8	3.9
13	3-OH-4-$OMeC_6H_3$	Dioxane–EtOH	251–253 dec	$C_{26}H_{18}O_8$	68.1	4.0	67.9	4.3
14	4-OH-3-$OMeC_6H_3$	Cyclohexanone	213–215	$C_{26}H_{18}O_8$				
15	2,4-$(OMe)_2C_6H_3$	EtOH–EtOAc	197–199	$C_{27}H_{20}O_8$	68.6	4.3	68.2	4.7
16	3,4-$(CH_2O_2)C_6H_3$		250 dec	$C_{26}H_{16}O_8$				

[a] Soluble in acid and alkali but could not be crystallized from any of the neutral solvents tried.

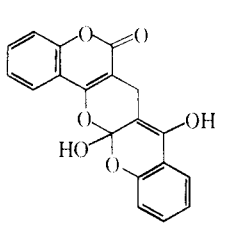
Later, Chmielewska and Cieslak[5] expressed the view that the activity could be correlated with antivitamin K property and the active form of a coumarin anticoagulant must be a cyclic ketal of the type II or III. These can arise only from 3-substituted 4-hydroxycoumarins in which the side chain has a carbonyl or a potential carbonyl group in position 2′ or 3′. The activity of dicoumarol was explained on the basis of its modified structure (IV) arrived at from methylation experiments; this would lead to the derived ketal V. An alternative possibility is the lactol structure VI arising from I. The recent suggestion of Lederer[6] that the form of vitamin K active in prothrombin synthesis is probably related to the structure VII lends support to Chmielewska's hypothesis.



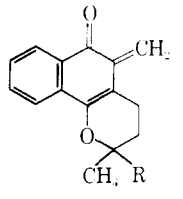
IV



V

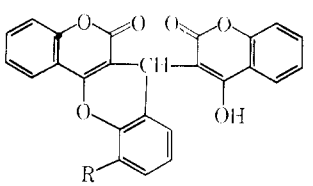


VI

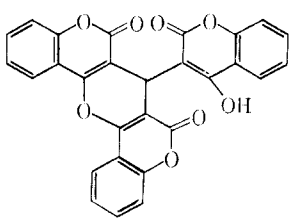


VII

Earlier, Link and co-workers[3] had examined some dicoumarols with a phenyl or a substituted phenyl group at the methylene bridge. We have now made more compounds of this type and have also included those described earlier for purposes of comparison. By condensing[7] 4-hydroxycoumarin with substituted benzaldehydes the following new dicoumarols have been prepared: *o*- and *p*-chloro-, *o*- and *p*-nitro-, *m*- and *p*-hydroxy-, *o*-, *m*-, and *p*-methoxy-, *p*-dimethylamino-, 3-hydroxy-4-methoxy-, and 2,4-dimethoxybenzylidenebis-4-hydroxycoumarins and 3,3′-cinnamylidenebis-4-hydroxycoumarin. Compounds having slightly modified structures have also been made. These are the products of condensation of 4-hydroxycoumarin and *o*-vanillin and the anhydrides obtained by dehydration of the corresponding 3,3′-benzylidenebis-4-hydroxycoumarins. The former has been given the structure IX on the basis of elemental analysis and in analogy with the earlier known product from salicylaldehyde (VIII). Attempts to condense 4-hydroxycoumarin with phloroglucinaldehyde failed. A new tricoumarol derivative X has been obtained during an attempted formylation of 4-hydroxycoumarin using dimethylformamide (DMF) and phosphorus oxychloride. The intermediate in this reaction should be 3-formyl-4-hydroxycoumarin which, however, could be isolated only as its 2,4-dinitrophenylhydrazone and as a minor product. The formation of X indicates ready condensation of the aldehyde with 4-hydroxycoumarin followed by dehydration. The structure X is supported by elemental analysis of the compound and of its acetate as well as



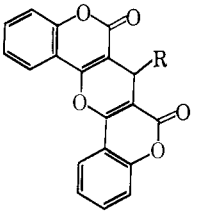
VIII, R = H
IX, R = OCH_3



X

[5] I. Chmielewska and J. Cieslak, *Tetrahedron*, **4**, 135 (1958).
[6] E. Lederer, *Biochem. J.*, **93**, 459 (1964).
[7] W. R. Sullivan, C. F. Huebner, M. A. Stahmann, and K. P. Link, *J. Am. Chem. Soc.*, **65**, 2288 (1943).

TABLE II
ANHYDRIDES OF 3,3′-BENZYLIDENEBIS-4-HYDROXYCOUMARINS



No.	R	Mp, °C[a]	Formula	—Calcd, %— C	H	—Found, %— C	H
1	*o*-ClC_6H_4	>310	$C_{25}H_{13}ClO_5$	70.1	3.0	69.4	3.8
2	*p*-ClC_6H_4	>310	$C_{25}H_{13}ClO_5$	70.1	3.0	69.5	2.7
3	*o*-$NO_2C_6H_4$	>310	$C_{25}H_{13}NO_7$	68.3	3.0	67.9	3.3
4	*p*-$NO_2C_6H_4$	>310	$C_{25}H_{13}NO_7$	68.3	3.0	69.0	3.3
5	*m*-$OAcC_6H_4$	305–306	$C_{27}H_{16}O_7$	71.7	3.5	71.6	3.9
6	*o*-$OCH_3C_6H_4$	>310	$C_{26}H_{16}O_6$	73.6	3.8	73.7	3.6
7	*m*-$OCH_3C_6H_4$	308–310	$C_{26}H_{16}O_6$	73.6	3.8	74.0	4.2
8	*p*-$(CH_3)_2NC_6H_4$	305–307	$C_{27}H_{19}NO_5$	73.9	4.3	74.0	4.4
9	3-OAc-4-$OCH_3C_6H_3$	274–276 dec	$C_{28}H_{18}O_8$	69.7	3.8	69.4	4.2
10	2,4-$(OCH_3)_2C_6H_3$	248–250[b]	$C_{27}H_{18}O_7$	71.4	3.9	71.2	4.6
11	3,4-$(CH_2O_2)C_6H_3$	*c*	$C_{26}H_{14}O_7$	71.3	3.2	71.8	3.6

[a] Recrystallized from cyclohexanone. [b] Sintered at 238°. [c] Shrank at 295°, no melting up to 310°.

by the similarity of its ultraviolet spectrum to that of dicoumarol.

The ultraviolet spectra of the compounds are remarkably similar to that of simple dicoumarol; they give two characteristic absorption maxima, near 280 and 305 mμ and two minima, 250–255 and 290–295 mμ.

Experimental Section

The various 3,3′-benzylidenebis-4-hydroxycoumarins listed in Table I were prepared by adopting the procedure described by Sullivan, *et al.*[7] All of the melting points were determined in open capillary tubes and are uncorrected.

Interaction of 4-hydroxycoumarin (0.5 g) with *o*-vanillin (0.25 g) in ethanol gave (IX) which crystallized from dioxane as colorless needles (0.45 g): mp 276–278° dec; λ_{max}^{EtOH} (qualitative) 265, 305 mμ.

Anal. Calcd for $C_{26}H_{16}O_7 \cdot 0.5H_2O$: C, 69.5; H, 3.8. Found: C, 69.7; H, 3.9.

The acetate crystallized from cyclohexanone as colorless plates, mp 264–267°.

Anal. Calcd for $C_{28}H_{18}O_8$: C, 69.7; H, 3.8. Found: C, 69.7; H, 4.2.

Formylation of 4-Hydroxycoumarin. A.—Phosphorus oxychloride (3 ml) was added dropwise to DMF (30 ml) cooled to 10°. The mixture was kept for 15 min at room temperature to complete complex formation. 4-Hydroxycoumarin (3 g) was added to the pale pink solution; immediately after solution a solid (A) separated. It was insoluble in hot ethanol or DMF and could be obtained only as amorphous material (1.5 g): mp >300°; λ_{max}^{EtOH} 270 mμ (log ε 3.82), 300 mμ (log ε 3.71); λ_{min} 255 mμ (log ε 3.71), 285 mμ (log ε 3.70).

Anal. Calcd for $C_{28}H_{14}O_8$: C, 70.3; H, 2.9. Found: C, 70.0; H, 3.2.

The acetate did not melt below 300°. It crystallized from ethanol–ethyl acetate as needles.

Anal. Calcd for $C_{30}H_{16}O_9$: C, 69.2; H, 3.1. Found: C, 68.7; H, 3.4.

B.—A mixture of 4-hydroxycoumarin (1 g), DMF (10 ml), and $POCl_3$ (1 ml) was heated on a boiling-water bath for 10 min, kept overnight, and slowly added to 10% aqueous Na_2CO_3 (40 ml). The mixture was heated to boiling and cooled, and the brown solid that had separated was filtered. It did not melt up to 300° and was the same as the compound described under A. The alkaline solution was acidified, and the precipitate was collected, washed, and dried. It melted at 125–126° and decomposed at 244–245°. It gave tests for aldehydes, but attempts to recrystallize it from hot ethanol resulted in the high-melting product described above. It readily gave a 2,4-dinitrophenylhydrazone, mp 272–274°.

Anal. Calcd for $C_{16}H_{10}O_7N_4$: C, 51.9, H, 2.7. Found: C, 51.4; H, 3.1.

The anhydrides (Table II) were prepared by the procedure of Huebner, *et al.*[8]

Experimental Pharmacology.—The anticoagulant activity was studied by determining the prothrombin time in the rabbit by Quick's one-stage method as modified by Montigel and Pulver.[9] The doses were calculated on an equimolar basis using a 50-mg/kg dose of dicoumarol as the reference standard. The results are tabulated in Table III.

Results and Discussion

In agreement with Link and co-workers,[3] it is now found that 3,3′-benzylidenebis-4-hydroxycoumarin is much less active than dicoumarol. The introduction of substituents in the phenyl on the bridge has varying effects depending on their nature and on their position. The methoxyl is the most favorable and the dimethylamino the least. *para* substituents evoke the maximum response and the effect falls as the substituent is moved on to the *meta* and *ortho* positions.

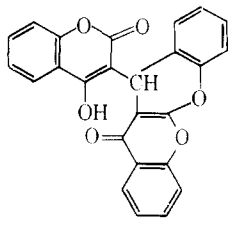
The formation of a ketal or a lactol would depend upon a proper steric disposition of the molecule. The unfavorable effect of the phenyl at the bridge may be due to distortion which is considerably enhanced in the case of the *ortho*-substituted phenyl derivatives. On the other hand, the product of condensation of 4-hydroxycoumarin with cinnamaldehyde, in which the phenyl is separated from the bridge by two carbons, has a much higher activity. Disubstitution as in the case of the products obtained with vanillin, isovanillin, 2,4-dimethoxybenzaldehyde, and piperonal lowers activity.

Compound VIII[7,10] is considerably more active than 3,3′-benzylidenebis-4-hydroxycoumarin. This result cannot be accommodated by the ketal hypothesis since VIII can yield only a lactol. It can, however, be explained if the structure is considered to be XI changing into a ketal form. It is not possible to decide between these two alternatives on the basis of available spectral and structure–activity relationships evidence.

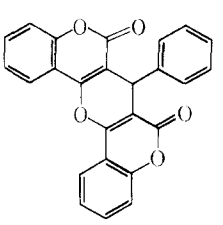
(8) C. F. Huebner, W. R. Sullivan, M. A. Stahmann, and K. P. Link, *J. Am. Chem. Soc.*, **65**, 2292 (1943).
(9) C. Montigel and R. Pulver, *Schweiz. Med. Wochschr.*, **82**, 132 (1952).
(10) F. Litvan and W. G. Stoll, *Helv. Chim. Acta*, **42**, 878 (1959).

TABLE III
ANTICOAGULANT ACTIVITY OF DICOUMAROLS

No.	Derivative of 4-hydroxycoumarin	Onset, hr	Action peak, hr	Duration, hr	Coagulation valency, %
1	Dicoumarol (ref compd)	24	120	216	0
2	3,3'-Benzylidenebis-	24	48	72	20
3	3,3'-(*o*-Chlorobenzylidene)bis-	24	48	96	35
4	3,3'-(*p*-Chlorobenzylidene)bis-	24	48	120	10
5	3,3'-(*o*-Nitrobenzylidene)bis-	24	48	144	20
6	3,3'-(*p*-Nitrobenzylidene)bis-	24	24	72	20
7	3,3'-(*m*-Hydroxybenzylidene)bis-		Inactive		100
8	3,3'-(*p*-Hydroxybenzylidene)bis-	24	48	72	25
9	3,3'-(*o*-Methoxybenzylidene)bis-	24	48	72	20
10	3,3'-(*m*-Methoxybenzylidene)bis-	24	48	120	10
11	3,3'-(*p*-Methoxybenzylidene)bis-	24	48	96	5
12	3,3'-(*p*-Dimethylaminobenzylidene)bis-	24	48	72	35
13	3,3'-Cinnamylidenebis-	24	48	144	10
14	3,3'-(3-Hydroxy-4-methoxybenzylidene)bis-	24	48	144	15
15	3,3'-(4-Hydroxy-3-methoxybenzylidene)bis-	24	48	72	20
16	3,3'-(2,4-Dimethoxybenzylidene)bis-	24	72	86	20
17	3,3'-(3,4-Methylenedioxybenzylidene)bis-	24	48	72	15
18	3-[6-Oxo-(1)-benzopyrano[4,3-*b*]-(1)-benzopyran-7-yl]-	24	48	144	5
19	3-[6-Oxo-(1)-benzopyrano[4,3-*b*]-(1)-11-methoxybenzopyran-7-yl]-	24	48	96	35
20	Anhydride of 3,3'-(*p*-methoxybenzylidene)bis-		Inactive		100
21	4-Hydroxycoumarinyl-2,3,5,6-(3',4',3'',4''-dicoumarinopyran-2,5-diene)		Inactive		100



XI



XII

The product of formylation of 4-hydroxycoumarin mentioned earlier is an anhydride and is analogous to the compounds (*e.g.*, XII) prepared by subjecting the various substituted dicoumarols to dehydration. These are inactive.

A significant feature of X and XII is their similarity in shape to the hypothetical ketal from dicoumarol VI. However, there is a difference, namely, the absence of the ketal group in the former, and this seems to lead to inactivity. If, however, the active form of dicoumarol is the lactol VII, the inactivity of the anhydrides can be explained merely by gross structural differences. Further studies are necessary to define the exact structure of the active form.

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Leguminosae Alkaloids. II. Alkaloids of *Lupinus westianus* Small[1]

STANLEY I. GOLDBERG AND MOHAMMAD S. SAHLI[2]

Department of Chemistry, University of South Carolina, Columbia, South Carolina 29208

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As part of our continuing program of examination of alkaloids elaborated by previously uninvestigated or partially investigated plant species of genera belonging to the family *Leguminosae*, we now report the results of our study of the plant, *Lupinus westianus* Small.[3] The only mention of this plant in the chemical literature is that due to Wall and co-workers,[4] who had included it in a large plant survey. Apart from noting the presence of alkaloids in *L. westianus*, however, Wall, *et al.*,[4] did not carry out any chemical investigations.

The plant material used in the present study was collected during the spring of 1962 near Panama City, Fla.[5] The extraction procedure and the methods used for separation and identification of the alkaloids are detailed in the Experimental Section. As it turned out, the three alkaloids elaborated by *L. westianus*, (−)-sparteine, (−)-lupinine, and (−)-multiflorine, constituting 0.16, 0.09, and 0.52% of the weight of the moisture-free plant, respectively, are all known substances. However, a mixture containing the three components, sparteine, lupinine, and multiflorine in 21, 16, and 63 mole %, respectively, was examined for physiological activity in two specific tests.

In the spontaneous activity test, the mixture was injected (intraperitoneal) into Swiss-Webster mice at dosage levels of 1, 5, and 50 mg/kg. Each dosage level was administered to four groups of 5 mice. The mice were placed in a photocell activity cage 1 hr after drug administration, and a 15-min test interval was measured. An identical procedure was followed with a control (saline solution) group of mice. The ratio

(1) For Part I see, S. I. Goldberg and R. F. Moates, *Phytochemistry*, in press.

(2) Taken in part from the dissertation submitted by M. S. S. in partial fulfillment of the Graduate School requirements for the Ph.D. in chemistry, University of South Carolina.

(3) Some taxonomists, as does G. H. M. Lawrence ("Taxonomy of Vascular Plants," The Macmillan Co., New York, N. Y., 1951, p 545 ff), classify the genus *Lupinus* under the subfamily *Lotoideae* or *Papilionoideae*.

(4) M. E. Wall, J. W. Garvin, J. J. Willaman, Q. Jones, B. G. Schubert, and R. A. Davidson, *J. Pharm. Sci.*, **50**, 1001 (1961).

(5) We are indebted to Professor Robert Godfrey of the Florida State University for his expert identification of *L. westianus* and for his aid in arranging for collection and shipments of the plants.