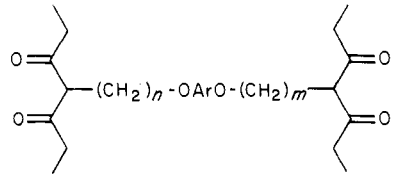
Antiviral Activity of Some β-Diketones. 3. Aryl Bis(β-diketones). Antiherpetic Activity[1]

Guy D. Diana,* Philip M. Carabateas, U. Joseph Salvador, Gordon L. Williams, Ethel S. Zalay, Francic Pancic, B. A. Steinberg, and Joseph C. Collins

Sterling-Winthrop Research Institute, Rensselaer, New York 12144. *Received December 2, 1977*

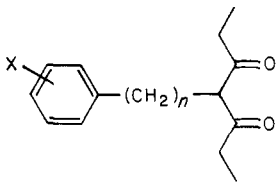
A series of bis(β-diketones) was synthesized and tested in vitro for antiviral activity against herpes simplex type 2. Two parameters which were studied in an effort to optimize activity were the nature of the aryl group and the length of the alkyl bridge. One of the more active compounds, 4,4′-[(1,4-phenylenedioxy)bis(6,1-hexanediyl)]-bis[3,5-heptanedione] (**6**), was evaluated more extensively and found to inhibit the cytopathic effect in tissue culture of herpes simplex virus type 1 as well as type 2. Compound **6** was evaluated in vivo topically against herpes simplex type 1 in experimentally induced skin infections in guinea pigs. A topical treatment with 2% of **6** in a vanishing cream base, administered 24 h postinfection applied five times daily for 4 days, significantly reduced the number and size of herpetic vesicles.

In continuation of our synthetic program aimed at preparing β-diketones with potential broad-spectrum antiviral activity, we have synthesized a series of aryl bis(β-diketones) with the general structure I. This work

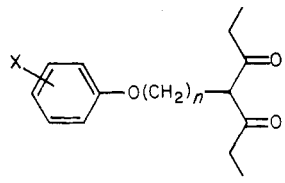


I

resulted from the discovery that a series of related compounds (II and III) exhibited a high degree of antiviral



II

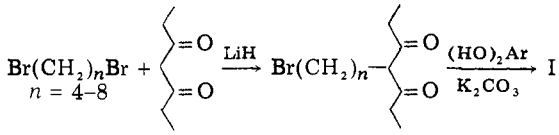


III

activity against both RNA and DNA viruses.[2-5] These compounds were particularly effective against herpes virus in vivo when applied as an ointment or in a Me_2SO solution to guinea pig skin infected with herpes simplex types 1 and 2.[6]

Chemistry. The majority of the compounds, with the exception of **1–4**, were prepared from the bromoalkyl diketone IV and the appropriate phenols as outlined in method A. Compounds **1** and **3** were synthesized ac-

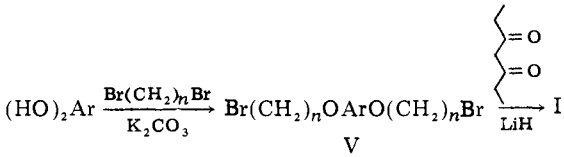
method A



IV

cording to method B. A similar approach, method C, was

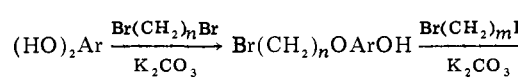
method B



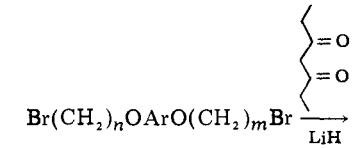
V

used for the preparation of the unsymmetrical bis(diketones) **2** and **4**. In the latter method, 1 equiv of the

method C



VI



VII

dibromide was used, and, as expected, the yield was only fair. The final alkylation was performed with lithium hydride in DMF.

Biological Results. The in vitro antiviral activity of the bis(diketones) against herpes virus was determined according to the procedure previously described.[2] The effect of varying the length of the alkyl bridge on the antiviral activity is demonstrated with compounds **1–8**. It is interesting to note that the two unsymmetrical diketones **2** and **4** were inactive at the highest levels tested. No activity was observed until the length of both alkyl groups was increased to 4 (compound **3**). The activity was sustained through compound **8** where $n = m = 8$, although a gradual decline was observed at this point. We chose to maintain the hexyl bridge, since compound **6** did prove to be significantly effective against other viruses, and proceeded to alter the phenyl group. Substitution with one or two chlorine atoms (compounds **10** and **11**, respectively) did not alter the activity of the parent compound **6** to a significant degree, although the latter two compounds did appear to demonstrate cytotoxicity at lower levels. The introduction of a methyl group (**12**) decreased activity.

Compound **6** was evaluated further against herpes virus types 1 (Sheely strain) and 2 (Curtis strain) in the plaque inhibition test and the results are shown in Table I. A significant reduction in plaque count was obtained against both viruses with a definite dose response being observed. At 4 μg/mL, compound **6** showed a 93 and 89% reduction in plaque count when tested against herpes virus types 2 and 1, respectively.

The antiherpetic activity of **6** was demonstrated in vivo in experimentally induced skin infections in guinea pigs. The guinea pigs were infected with herpes virus type 1 by means of a vaccination gun which was used to make multiple skin punctures in a delineated area of the shaved and epilated flanks in the presence of 0.1 mL of a tissue culture preparation with a titer of $10^{6.25}$ $TCID_{50}$. Compound **6** was prepared in a vanishing cream base and administered topically starting 24 h postinfection, five

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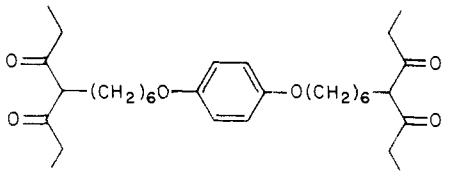
Table I. Plaque Inhibition of Herpes Simplex Virus Type 2 (Curtis Strain) and Type 1 (Sheely Strain) in BSC-1 Cell Culture (Monkey Kidneys)



6

μg/mL	Virus	Plaque count (av)					% inhibn
2	HSV-2 Curtis	18	17	17	19	(18)	18
3	HSV-2 Curtis	10	8	10	9	(9)	59
4	HSV-2 Curtis	2	2	0	2	(1.5)	93
	Virus control	20	19	23	27	(22)	
2	HSV-1 Sheely	45	43	42	45	(44)	21
3	HSV-1 Sheely	39	40	38	10	(32)	43
4	HSV-1 Sheely	2	6	12	3	(6)	89
	Virus control	60	56	43	65	(56)	

Table II. Statistical Analysis of the Effect of 2% of Compound 6 in a Vanishing Cream Base Applied Five Times Daily for 4 Days on the Development of Herpetic Vesicles in Guinea Pigs Infected Intradermally with Herpes Simplex Virus Type 1, AA Strain[a]

Day postinfection	Mean value of vesicle scores			
	Placebo		41 908, 2%	
	P[c]	S[d]	P	S
2[b]	1.1	1.3	0.8	0.8[e]
3	2.5	1.7	1.0[f]	0.9[e]
4	2.7	2.4	1.0[f]	1.3[e]
5	2.5	2.5	0.8[f]	0.7[f]
7	2.1	2.0	0.3[f]	0.6[f]

[a] Treatment started 24 h postinfection. [b] 24 h after treatment was initiated. [c] Scorer 1. [d] Scorer 2. [e] Significantly different from corresponding placebo mean, $p = 0.05$. [f] Significantly different from corresponding placebo mean, $p \leq 0.01$.

times daily for 4 days. The lesions were scored on a scale of 0–3+ with 0.5 increments on a blind basis by two independent scorers who judged the severity of the herpetic lesions by the size and number of herpetic vesicles. At the end of 7 days, the scores were analyzed by the Fisher-Behren's "T" test to determine their significance. Within 48 h (24 h after the first medication), a 2% preparation of compound **6** produced a sharp reduction in vesicles score which represented a significant reduction in the number and size of the herpetic vesicles when compared to the placebo-treated animals (Figure 1). While the severity of herpetic lesions increased in the placebo-treated animals, peaking between days 3 and 5, animals treated with **6** exhibited only minimal lesions throughout the course of infection with drying of vesicles and scab formation observed after 4 days postinfection. Although the medication was discontinued after 4 days, no relapse of infection was observed in the group of animals by day 7 when only a few scabs remained. Scab formation in the placebo-treated animals was observed only after 7 days.

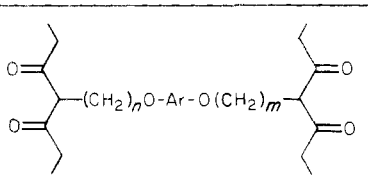
The statistical analysis of the data is shown in Table II. A significant difference between drug and placebo-treated animals was detected by both observers on day 3 postinfection or after 48 h of treatment and persisted throughout the course of the infection.

Discussion

The in vitro data summarized in Table III seem to indicate the necessity of a proper hydrophilic–lipophilic balance for optimum activity. The inactivity of the unsymmetrical homologues **2** and **4** is at the present time surprising and unexplainable since we had anticipated that the activity of **4** would be comparable to or greater than compound **3**. Although compound **6** did not demonstrate the highest level of activity in vitro, it proved to be extremely effective when tested in vivo. This phenomenon was observed in other series[2,3] and emphasizes the fact that the levels of in vitro activity do not necessarily carry over to the in vivo systems.

Among other antiviral agents, only phosphonoacetic acid has been reported to be effective against herpes virus infections of the skin in experimental animals when applied topically as an ointment.[7] Idoxuridine has been

Table III



Compd	Ar	n	m	Mp or bp (mm), °C	Method	% yield[b]	Formula[c]	MIC,[a] μg/mL, HSV[d] type
1	1,4-C_6H_4	3	3	57–58[e]	B	15.1	$C_{26}H_{38}O_6$	Inactive
2	1,4-C_6H_4	3	4	*f*	C	10.8	$C_{27}H_{40}O_6$	Inactive
3	1,4-C_6H_4	4	4	51–52[g]	B	24.3	$C_{28}H_{42}O_6$	6-3
4	1,4-C_6H_4	4	5	*h*	C	30	$C_{29}H_{44}O_6$	Inactive
5	1,4-C_6H_4	5	5	*i*	A	8.5	$C_{30}H_{46}O_6$	6-3
6	1,4-C_6H_4	6	6	56–57[e]	A	16.7	$C_{32}H_{50}O_6$	50-6
7	1,4-C_6H_4	7	7	56–57[j]	A	20.8	$C_{34}H_{54}O_6$	50-25
8	1,4-C_6H_4	8	8	52–54[e]	A	53.1	$C_{36}H_{58}O_6$	Inactive
9	1,3-C_6H_4	6	6	*k*	A	52.2	$C_{32}H_{50}O_6$	50–12
10	1,4-(2-Cl)C_6H_3	6	6	260–265 (0.01)	A	26.6	$C_{32}H_{49}ClO_6$	12-6
11	1,4-(2,5-Cl_2)C_6H_2	6	6	*k*	A	41.6	$C_{32}H_{48}Cl_2O_6$	12-6
12	1,4-(2-CH_3)C_6H_3	6	6	250–255 (0.005)	A	43.2	$C_{33}H_{52}O_6$	50-25

[a] Minimum inhibitory concentration. [b] Yields refer to analytically pure product. [c] Analyzed for C, H, and Cl where applicable. [d] Herpes virus. [e] Recrystallized from ether. [f] Pure sample obtained by column chromatography on Florisil and eluted with ether-benzene (40:60). [g] Recrystallized from 2-propanol. [h] Pure sample obtained by liquid-phase chromatography on silica gel and eluted with toluene–ethyl acetate (90:10). [i] Pure sample obtained from column chromatography on silica and eluted with chloroform–methanol (95:5). [j] Recrystallized from methanol. [k] Purified by column chromatography on silica gel and eluted with ether–hexane (50:50, compound **9**) and hexane–ethyl acetate (80:20, compound **11**).

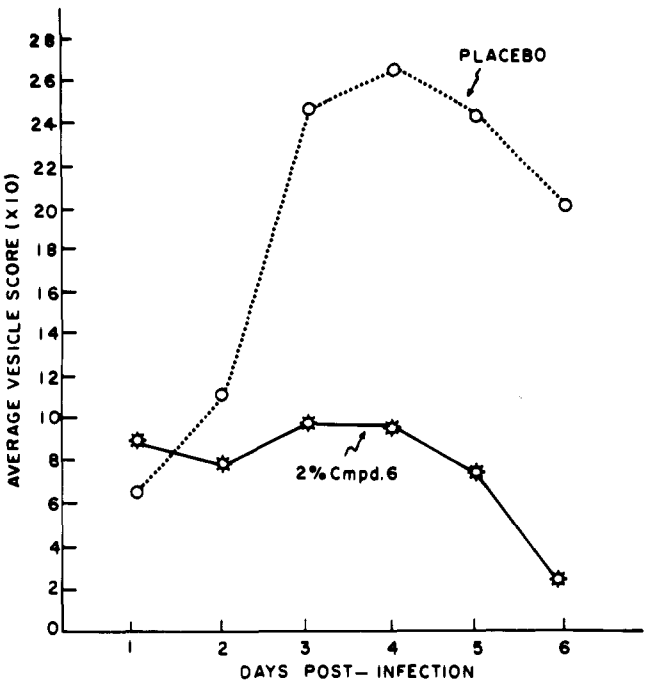


Figure 1. The effect of compound 6 in cream base on the development of herpetic vesicles in the guinea pig infected intradermally with herpes virus hominus, type 1, AA strain; medication given topically five times daily for 4 days.

found effective against varicellazoster infections when applied as a 40% solution in Me_2SO.[8] Consequently, we are pursuing this approach to the treatment of herpetic infections of the skin.

Experimental Section

Melting points were run according to the USP procedure and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results are within +0.4% of the theoretical values. Analyses were performed by Instranal Laboratories, Rensselaer, N.Y. NMR spectra were determined on a Varian A-60 spectrophotometer and the mass spectra on a Jeolco double-focusing high-resolution mass spectrometer by R. K. Kullnig and S. Clemans.

Plaque Reduction Assay of Herpes Simplex Virus Types 1 and 2. Four-day-old confluent monolayers of BSC-1 (monkey kidney cells) in 30-mL Folcon flasks were used. Nutrient medium was removed and cultures were infected with 20–60 plaque-forming units of herpes simplex virus contained in 0.5 mL of tissue culture medium (MEM) + 2% fetal calf serum. Quadruplicate infected monolayers were overlaid with semisolid agar containing equal volumes of 1% ionogar + 2 × M-199 medium + 5% fetal calf serum. Specific concentrations of compound 6 to be tested were incorporated into the agar overlay. As controls, uninfected monolayers were overlaid with medium containing various concentrations of 6. Cultures were incubated in an inverted position at 37 °C. After 4 days incubation cultures were fixed in 10% formalin (in 2% sodium acetate) overnight at 4 °C. The fixed cells were stained with crystal violet in formalin. Plaques, i.e., those areas in which virus activity has destroyed cells and thus are unstained, were counted in each flask and the average count for four culture flasks was established. The percentage of plaque reduction in drug-treated vs. infected nontreated controls was established.

Preparation of Bis(diketones). Method A. 4,4′-[(1,4-Phenylenedioxy)bis(6,1-hexanediyl)]bis[3,5-heptanedione] (6). A mixture of 2.74 g (0.025 mol) of hydroquinone, 17.5 g (0.06 mol) of 4-(6-bromohexyl)-3,5-heptanedione,[2] 20.6 g (0.15 mol) of K_2CO_3, 2.0 g of KI, and 150 mL of butanone was heated to reflux and left for 28 h. After cooling, the solid was removed by filtration and washed with butanone and the filtrate concentrated to dryness. The residual oil was dissolved in methylene dichloride and the undissolved solid removed by filtration and discarded. After removal of the solvent, the residual oil was distilled at 235–245 °C (0.03 mm) giving 12 g of an oil. This material was then further purified by column chromatography on silica and eluted with 4:1 hexane–ethyl acetate to give a solid which, after recrystallization from ether–pentane, gave 2.2 g (16.7%), mp 56–57 °C. Anal. ($C_{32}H_{50}O_6$) C, H.

Method B. 1,4-Bis(4-bromobutoxy)benzene (V, n = 4). To a solution of 11.6 g (0.281 mol) of NaOH (97%) in 250 mL of ethanol was added at room temperature 14.2 g (0.129 mol) of hydroquinone in 50 mL of ethanol. The solution was stirred at room temperature for 15 min and then heated to 40 °C for 0.5 h. After cooling to 4 °C 168 g (0.780 mol) of 1,4-dibromobutane was added and the solution was allowed to come to room temperature and left for 6 h. Finally the solution was heated to reflux for 18 h. The resulting mixture was then concentrated in vacuo and the residue partitioned between methylene dichloride and water. The organic layer was washed and dried and removal of the solvent gave an oil. The excess dibromobutane was removed in vacuo and a solid resulted. Recrystallization from methanol gave 21.6 g (44%), mp 89–91 °C. Anal. ($C_{14}H_{20}Br_2O_2$) C, H, Br.

4,4′-[(1,4-Phenylenedioxy)bis(4,1-butanediyl)]bis[3,5-heptanedione] (3). To a suspension of 1.79 g (0.226 mol) of LiH in 185 mL of DMF was added dropwise over a 20-min period 31.8 g (0.248 mol) of 3,5-heptanedione. The reaction temperature rose to 63 °C. The mixture was warmed on a steam bath to 80–85 °C for 1 h and then stirred for an additional hour at room temperature. Then the dibromide V (n = 4), 21.5 g (0.05 mol), was added and the solution was heated to 70 °C for 2 days. The solution was poured into 925 mL of water, 450 mL of water and 60 mL of concentrated HCl added, and the resulting mixture extracted with ether. After washing and drying, the ethereal layer was concentrated in vacuo and the unreacted dione was removed by distillation [bp 65–68 °C (15 mm)]. The resulting solid was recrystallized from 2-propanol: 6.5 g (24.3%); mp 51–52 °C. Anal. ($C_{28}H_{42}O_6$) C, H.

Method C. 4-(4-Bromobutoxy)phenol (VI, n = 4). A solution of 110 g (1 mol) of hydroquinone, 56 g (1 mol) of KOH, and 215 g (1 mol) of 1,4-dibromobutane in 1200 mL of methanol was heated to reflux for 5 h. After cooling, the precipitated solid was removed by filtration and the filtrate concentrated to dryness. The residual solid was partitioned between ether (500 mL) and 1 L of water. After drying, the ethereal layer was concentrated to dryness leaving 225 g of oil which solidified. This material was stirred with 1200 mL of benzene and the undissolved material separated and discarded. The solution was again concentrated in vacuo and the unreacted dibromobutane removed at 115 °C (1 mm). The remaining solid as recrystallized from 800 mL of benzene and 1200 mL of pentane to give 80.6 g (33%), mp 68–70 °C. Anal. ($C_{10}H_{13}BrO_2$) C; Br: calcd, 32.60; found, 31.91. H: calcd, 5.34; found, 5.81.

1-(4-Bromobutoxy)-4-(4-bromopropoxy)benzene (VII, m = 3, n = 4). A mixture of 24.5 g (0.1 mol) of VI, 22.0 g (0.12 mol) of 1,3-dibromopropane, 16.8 g (0.12 mol) of K_2CO_3, 1 g of KI, and 600 mL of butanone was heated to reflux for 24 h. The mixture was filtered hot and the filtrate concentrated to dryness. The residual oil was partitioned between methylene dichloride and water and the organic layer washed and dried. Removal of the solvent gave a solid which after recrystallization from pentane gave 31 g (85%) of product, mp 64–65 °C. Anal. ($C_{13}H_{18}Br_2O_2$) C, H, Br.

References and Notes

(1) Presented in part at the 10th International Congress of Chemotherapy, Zurich, Switzerland, Sept 18–23, 1977.

(2) G. D. Diana, U. J. Salvador, E. S. Zalay, R. E. Johnson, J. C. Collins, D. Johnson, W. B. Hinshaw, R. R. Lorenz, W. H. Thielking, and F. Pancic, *J. Med. Chem.*, **20**, 750 (1977).

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(4) G. D. Diana, R. Johnson, U. J. Salvador, E. Zalay, P. Carabateas, G. Williams, and F. Pancic, paper presented at the 15th National Chemistry Symposium, Salt Lake City, Utah, June 20–24, 1976.

(5) F. Pancic, J. Huang, M. Seipel, M. Feth, D. Carpentier, and W. Goss, paper presented at the 16th Interscience Con-

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trans-2,3b,4,5,7,8b,9,10-Octahydronaphtho[1,2-*c*:5,6-*c*']dipyrazole, a New Orally Active Antiallergic Compound

María J. Vítolo, Víctor E. Márquez,*[1]

Departamento de Investigación, Laboratorio de Química, Laboratorios Cosmos S.A., Caracas 106, Venezuela

and Inés Hurtado

Laboratorio de Alergia Experimental, Centro de Microbiología y Biología Celular, Instituto Venezolano de Investigaciones Científicas, Caracas 101, Venezuela. *Received September 9, 1977*

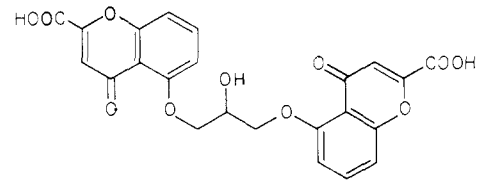
The synthesis and antiallergic activity of a new heterocyclic steroidal molecule are described. Compound 1 was shown to inhibit the rat passive cutaneous anaphylaxis and its activity in this system was compared to that of disodium cromoglycate. It is orally active at a dose of 35 mg/kg (ED_{50}) and its activity persists for up to 6 h for larger doses.

The search for active inhibitors of mediator release in sensitized tissues is a field that has received a great deal of attention since the discovery of disodium cromoglycate (DSCG).[2] Active drugs in this area, of which DSCG is the prototype, do not appear to interfere with antigen–antibody association, but rather they seem to inhibit the process by which such association initiates the release of chemical mediators.[3] Recent cell binding studies carried out with [^{3}H]-DSCG suggest that the site of action of DSCG is at or near the surface of the target cell membrane.[4] Likewise, the reported inhibition of hypersensitivity reactions in humans by the pentapeptide Asp-Ser-Asp-Pro-Arg, which displaced IgE from its binding sites on dermal target cells, suggests that this inhibitor also acts at or near the cell membrane.[5] It seems, therefore, that membrane selectivity is necessary for some substances to behave as inhibitors of anaphylaxis.

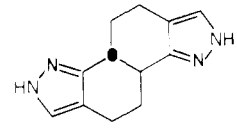
When compound 1 was found to have activity against passive cutaneous anaphylaxis (PCA), it was of interest to consider whether or not its steroid-like structure was in any way responsible for the observed activity. Although steroids do not appear to have a direct effect on the release of mediators of anaphylaxis, they are known to interact with membranes and promote biomembrane stabilization.[6,7] In addition, pyrazoles fused to a steroid A ring are known to enhance their antiinflammatory activity.[8] Another feature of compound 1 worth considering was the symmetric disposition of the pyrazole rings. Symmetry does not appear to be a prerequisite for anti-PCA activity as shown by the fact that there are many active molecules that are nonsymmetric.[9] However, a symmetric molecule may provide some information about the distance between active sites on the receptor even if both functional groups are involved in drug–receptor interactions that may differ in significance.

The synthesis and biological activity of compound 1 are the subject of the present paper.

Chemistry. The synthetic sequence started with commercially available 1,5-decalindiol (**2**, mixture of isomers) which was subjected to oxidation under the conditions reported by Johnson et al.[10] (Scheme I). As reported by the same authors, the resulting dione was equilibrated to its more stable trans isomer and obtained as pure *trans*-decalin-1,5-dione (**3a**). Treatment of **3a** with ethyl formate in pyridine utilizing sodium methoxide as catalyst afforded intermediate **4** which according to NMR appears to be a mixture of rapidly equilibrating tautomers (**4a** and **4b**).[11] NMR spectral comparison with *cis*- and *trans*-decalin-1,5-dione established that compound **4** had the trans configuration.[11] Furthermore, compound **4** was also obtained starting with pure *cis*-decalin-1,5-dione, thus eliminating the need to purify and separate the mixture of diones **3a** and **3b**. Final product **1** was obtained also as a mixture of pyrazole tautomers in good yield from the reaction of **4** with hydrazine in refluxing ethanol. In a similar fashion compounds **5** and **6** were obtained from **4** when treated with methylhydrazine and phenylhydrazine, respectively. According to Cospeau and Elguero,[12] the keto enol equilibria in hydroxymethylene ketones and the two nucleophilic nitrogens of monosubstituted hydrazines could give rise to two isomers. In the case of methylhydrazine the major component of the mixture appeared to be compound **5** which resulted from the attack of the most nucleophilic alkyl nitrogen on the exocyclic carbonyl carbon of compound **4b**. Purification of **5** was accomplished after several recrystallizations from acetone. When phenylhydrazine was employed the nucleophilicity of the nitrogens was reversed and only one product was isolated. Structure **6** is proposed based on previous findings that state that hydroxymethylene ketones cyclize with arylhydrazines to give exclusively the N(1) derivative.[13]



DSCG



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Biological Activity and Discussion. Compound 1 inhibited the passive cutaneous anaphylaxis (PCA) reaction when administered per os 1–6 h before antigen challenge. The characteristics of the inhibitory effect of compound 1 are summarized in Table I. PCA reactions

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