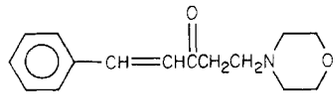
Mannich Bases of 4-Phenyl-3-buten-2-one: A New Class of Antiherpes Agent

M. L. Edwards,* H. W. Ritter, D. M. Stemerick, and K. T. Stewart

Merrell Research Center, Merrell Dow Pharmaceuticals Inc., Subsidiary of The Dow Chemical Company, Cincinnati, Ohio 45215. *Received May 14, 1982*

The morpholine Mannich base of 4-phenyl-3-buten-2-one was found to have activity in model infections of herpes simplex. The activity was essentially equivalent to that of disodium phosphonoacetate. This paper presents the biological activity and synthesis of a series of derivatives and analogues of the lead compound.

Infection by Herpes virus hominis (HVH) is a medical problem for which, at present, there is no satisfactory treatment available.[1] In the course of a general screening program, compound **1**, a Mannich base of 4-phenyl-3-buten-2-one, was found to have useful activity in a model HVH infection. This activity was observed for infections by types I and II of the virus. We have prepared a number of analogues of compound **1**, and in this paper we report the synthesis and structure–activity relationships of the series.



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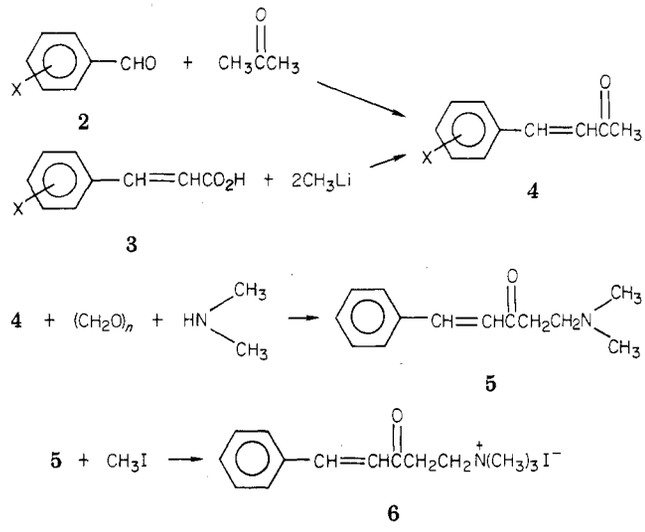
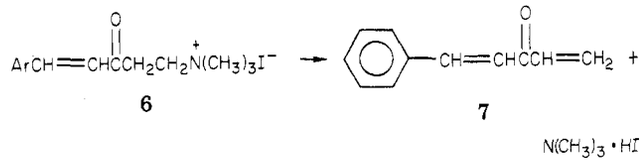
Chemistry. The route used for the synthesis of the majority of the compounds is given in Scheme I. Substituted 4-phenyl-3-buten-2-ones (**4**) were available by Claisen–Schmidt condensation of the appropriate benzaldehyde with acetone or by reaction of a cinnamic acid with 2 equiv of methyllithium.[2] Compounds **4** were reacted with dimethylamine hydrochloride or morpholine under standard Mannich conditions to give the products **5**. The dimethylamine Mannich bases were converted to the quaternary compounds **6** by reaction with methyl iodide in ether. The quaternary compounds required careful handling to isolate in pure form due to an elimination reaction to the dienone **7** (Scheme II).[3] Recrystallization of compound **6**, if heating was involved, invariably gave a product contaminated with trimethylamine hydriodide. Reaction of the morpholine Mannich bases with methyl iodide gave only recovered starting material, with no quaternary derivative being isolated.

The sulfur analogues were prepared by the route illustrated in Scheme III; the trimethylsilyl enol ether **8** was prepared from **4** by use of lithium diisopropylamide (LDA) and trimethylsilyl chloride in tetrahydrofuran.[4] Reaction of the trimethylsilyl ether **8** with chloromethyl methyl sulfide in methylene chloride in the presence of a catalytic amount of zinc chloride[5] gave the sulfide **9**. This compound was a liquid at room temperature and proved unstable when distillation was attempted. We were unable to obtain satisfactory elemental analyses of **9** due to the presence of small amounts of ketone **4**. Derivatives of **9** (**10**, **31**, and **44**) were crystalline and were fully characterized. Reaction of compound **9** with methyl iodide in ether gave the sulfonium salt **10**. Isolation of **10** also required care, as formation of the dienone **7** was observed on heating.

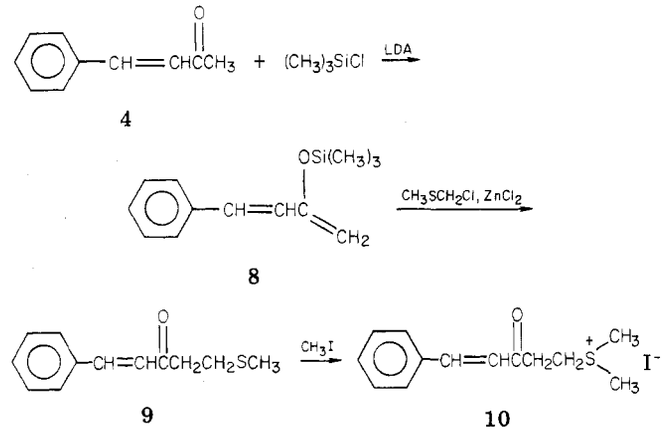
Scheme I



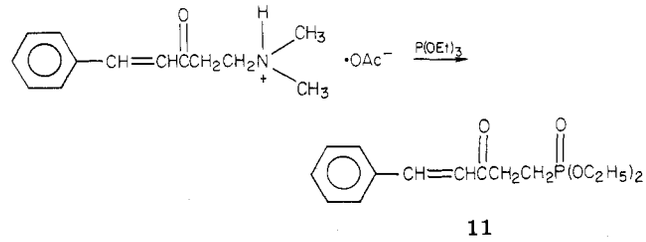
Scheme II



Scheme III



Scheme IV



(1) H. H. Handsfield and M. Turk, *Annu. Rep. Med. Chem.*, **14**, 114 (1979).
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(4) H. O. House, L. J. Czuba, M. Gall, and D. H. Olmstead, *J. Org. Chem.*, **34**, 2324 (1969).
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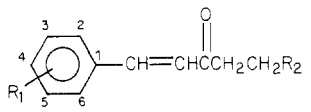
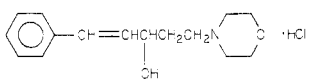
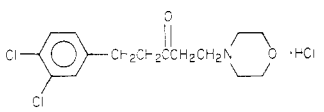
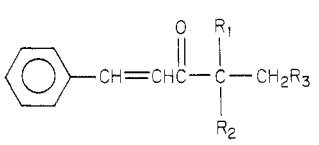
Chart I



	R_1	R_2
1	H	morph·HCl [a]
5	H	$N(CH_3)_2$·HCl
6	H	$N(CH_3)_3^+I^-$
9	H	SCH_3
10	H	$S(CH_3)_2^+I^-$
11	H	$P(=O)(OC_2H_5)_2$
12		
13		
14	3,4-Cl_2	morph·HCl
15	4-SCH_3	morph·HCl
16	4-SO_2CH_3	morph·HCl
17	3-CF_3	morph·HCl
18	2,6-Cl_2	morph·HCl
19	3,4-OCH_2O	morph·HCl
20	4-Cl	morph·HCl
21	4-CN	morph·HCl
22	4-OH	morph·HCl
23	3-OCH_3	morph·HCl
24	4-OCH_3	morph·HCl
25	4-CH_3	morph·HCl
26	3-NO_2	morph·HCl
29	H	S-C_6H_5
30	H	$S(n\text{-}C_3H_7)CH_3^+I^-$
31	H	$S(=O)CH_3$
44	H	SO_2CH_3
45	H	SO_2-C_6H_5
46	H	NH-C_6H_5
47	H	S-n-C_3H_7
48	2,6-Cl_2	SCH_3
49	2,6-Cl_2	$S(CH_3)_2^+I^-$
52	3-OCH_3	$N(CH_3)_3^+I^-$

[a] Abbreviated as morph·HCl.

Chart II



	R_1	R_2	R_3
27	CH_3	CH_3	$N(CH_3)_3^+I^-$
28	H	CH_3	$N(CH_3)_3^+I^-$
50	H	CH_3	$N(CH_3)_2$·HCl
51	CH_3	CH_3	$N(CH_3)_2$·HCl

The sulfoxide **31** was prepared from **9** with sodium metaperiodate; the sulfone **44** was prepared by reaction with 2 equiv of *m*-chloroperbenzoic acid in methylene chloride.

Reaction of chloromethyl phenyl sulfide with compound **8** gave the phenyl sulfide **29**. The methylsulfonium compound from **29** resisted characterization due to rapid decomposition. Oxidation of **29** with *m*-chloroperbenzoic acid gave sulfone **45**. The phosphonate **11** was prepared by reaction of the dimethylamino derivative (acetate salt) with triethyl phosphite (Scheme IV).[6]

(6) J. Szmuszkovicz, *J. Am. Chem. Soc.*, **80**, 3782 (1958).

Scheme V

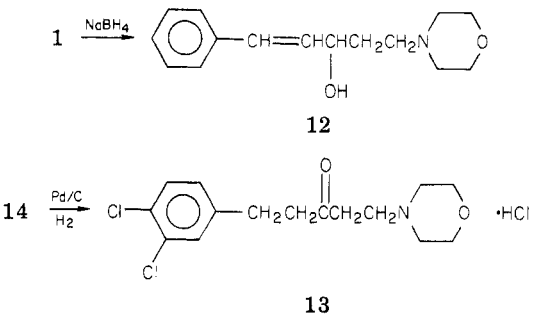
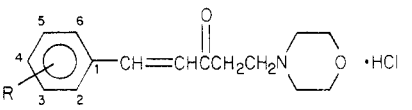


Table I. Antiherpes (Type 1) Activity of Nuclear-Substituted Analogues. Mouse Paralysis Model [a]



compd	R	% T/C [b]
1	H	11 [c]
14	3,4-Cl_2	0
15	4-SCH_3	12
16	4-SO_2CH_3	12
17	3-CF_3	11
18	2,6-Cl_2	14
19	3,4-OCH_2O	14
20	4-Cl	12
21	4-CN	24
22	4-OH	63
23	3-OCH_3	0
24	4-OCH_3	25
25	4-CH_3	38
26	3-NO_2	47
disodium phosphonoacetate, 1.5%		24
0.75%		61
untreated control [d]		95% paralyzed

[a] Compounds dissolved in 1.4% aqueous polyvinyl alcohol. Concentration of compound was 3%. [b] See Biological Methods section for detailed experimental method. % T/C = (percent of treated animals paralyzed)/(percent control (no drug) animals paralyzed) × 100. [c] Ten mice used for each compound. [d] Twenty mice per group: number is percent controls paralyzed, not % T/C.

Table II. Antiherpes (Type 1) Activity of Side Chain Modified Analogues. Mouse Paralysis Model [a]

compd [b]	% T/C [c]
1	11
12	88
13	50
control [d]	95% paralyzed

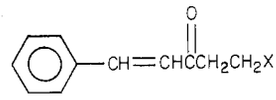
[a] See Biological Methods section for test procedure. [b] Compounds administered as 3% solution in 1.4% aqueous polyvinyl alcohol. Ten mice per compound. [c] See Biological Methods section for detailed experimental method. % T/C = (percent of treated animals paralyzed)/(percent control (no drug) animals paralyzed) × 100. [d] Control group contained 20 mice.

Reduction of the carbonyl ($NaBH_4$) and double bond (H_2, Pd/C) of **7** and **14**, respectively, gave compounds **12** and **13** in a straightforward manner (Scheme V).

Biological Methods.[7] Two in vivo models were used

(7) The test systems were developed at Merrell Dow by Dr. G. D. Mayer, Department of Chemotherapeutics, and have not been described previously.

Table III. Antiherpes (Type 1) Activity of Terminus-Modified Analogues. Hairless Mouse Model [a]



compd	X	av lesion score [b]	% reduction (from control) of lesion
1	$-N(CH_2CH_2)_2O\cdot HCl$	0.17	94
5	$N(CH_3)_2\cdot HCl$	1.8	30
6 [e]	$N^+(CH_3)^+I^-$	1.00	64
10	$S(CH_3)_2{}^+I^-$	0.5	82
11	$P(=O)(OC_2H_5)_2$	2.3	10
29	$S\text{-}C_6H_5$	2.5	10
30	$S(n\text{-}C_3H_7)CH_3{}^+I^-$	0.67	74
31	$S(=O)CH_3$	1.3	48
44 [f]	SO_2CH_3		
45 [f]	$SO_2\text{-}C_6H_5$		
46	$NH\text{-}C_6H_5$	2.67	9
47	$S\text{-}n\text{-}C_3H_7$	2.8	0
disodium phosphonoacetate [c]		0.33-0.67	74-88
control [d]		2.5-2.9	0

[a] See Biological Methods section for detailed experimental procedure. For structures, see Chart I. [b] Concentration of all compounds 3% in 1.4% aqueous polyvinyl alcohol. Six mice per group. [c] 5% concentration. [d] Twelve mice. Average lesion score is given as a range as not all compounds evaluated in same test. [e] Compound tested at 0.75% concentration. Higher concentrations were irritating. [f] Not evaluated in this test system; tested and found to be inactive in mouse paralysis model.

in the evaluation of the compounds. The first test was a mouse (CD-1, Charles River) paralysis model in which HVH-type I was applied topically. Scratches were made at the base of the tail, and virus was inoculated onto the scratched site by using sterile swabs. Experimental or reference compounds were applied 3 times to the infected site at 2, 4, and 6 h following infection. In untreated but infected mice a hindlimb paralysis occurred between 6 and 8 days postinfection. The data are presented as percent animals paralyzed treated/percent animals paralyzed control (% T/C) at the conclusion of the test (Tables I and II).

A secondary evaluation, by the same dose regimen described above, was performed with hairless mice. (These are from an in-house colony of hairless mice, originally from Jackson Labs.) In this model, several scratches were made in the lumbar region at the base of the tail, and HVH (types I or II) of known infectivity was applied to the scratched site. By the 8th day of infection, in untreated mice, severe ulcerated dermal lesions occurred. The severity of the developed lesion in each mouse was scored subjectively on a scale of 0 to 3, with 0 indicating no lesion and 3 indicating maximal lesion development.

Four compounds were evaluated in the hairless mouse model at a range of doses. These dose–response data are presented in Table IV. In all these tests, disodium phosphonoacetate was used as the reference compound. We were unable to demonstrate activity of our compounds when they were administered by the oral or subutaneous route.

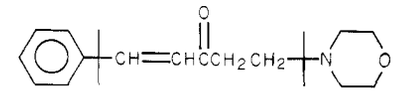
Table IV. Dose-Response of Selected Compounds. Hairless Mouse Test: Herpes Type 1 [a]

compd	dose, [b] %	av lesion score	% reduction in lesion severity
1	3	0.33	87
	1.5	0.55	80
	0.75	0.83	67
	0.37	0.83	67
14	3	0.5	80
	1.5	0.5	80
	0.75	0.5	80
	0.37	0.83	67
6	3	0.33	88
	1.5	0.50	82
	0.75	0.67	76
	0.37	0.67	76
10	3	0.33	88
	1.5	0.50	82
	0.75	0.67	76
	0.37	0.67	76
disodium phosphonoacetate	5	0.33	87
	3	0.83	69
	0.75	2.5	9
untreated control [c]		2.5-2.67	0

[a] See Biological Methods section for description of test. [b] Compounds administered at dose shown in 1.4% aqueous polyvinyl alcohol. Six mice per dose level. [c] Twelve mice in control. Score given as a range because not all compounds evaluated in the same test.

Results and Discussion

The structural variations performed were of three types, aromatic substitution (A), side chain modification (B), and



A B C

(8) J. M. Grisar, G. P. Claxton, K. T. Stewart, R. D. MacKenzie, and T. Kariya, *J. Med. Chem.*, **19**, 1195 (1976).
(9) R. W. Turner and T. P. Seden, German Offen. 2413233 (1974); *Chem. Abstr.*, **82**, 4272 (1975).
(10) A. McGookin and I. M. Heilbron, *J. Chem. Soc.*, **125**, 2099 (1924).
(11) I. M. Heilbron and R. Hill, *J. Chem. Soc.*, 2863 (1928).
(12) L. A. Jones and R. L. Johnston, *J. Chem. Eng. Data*, **16**, 112 (1971).
(13) J. S. Buch and I. Mmheilbron, *J. Chem. Soc.*, **121**, 1100 (1922).
(14) J. Suwinski and C. Troxzkiewicz, *Zesz. Nauk. Politech. Slask., Chem.*, 43 (1967); *Chem. Abstr.*, **69**, 72460 (1968).

Table V. Antiherpes (Type 1) Activity in Hairless Mouse Model [a]

compd	av lesion score [b]	% reduction from control of lesion
1	0.17	94
5	1.8	30
6 [c]	1.0	64
28	0.33	89
27	2.33	13
52	0.33	86
untreated control [d]	2.6-2.9	0

[a] See Biological Methods section for description of test. [b] Compounds administered topically as a 3% solution in 1.4% aqueous polyvinyl alcohol. Six mice per group. [c] Compound is irritating at this dose. [d] Twelve mice. Average lesion score is given as a range, since not all compounds evaluated in the same test.

Table VI. Comparison of Compound 1 and Disodium Phosphonoacetate vs. HVH Type 2 in Hairless Mice [a]

compd	concn, [b] %	% reduction in lesion severity
1	2	85
	1.5	78
	0.75	78
	0.37	70
disodium phosphonoacetate	5	78

[a] See Biological Methods section for detailed description of test procedure. [b] Compounds administered topically at concentrations shown in 1.4% aqueous polyvinyl alcohol. Six mice per dose level.

terminus modification (C). The analogues with aromatic substitution variations are presented in Table I. Most of these compounds were as active (low % T/C values) as the lead compound (1). We adopted the premise that formation of the dienone **7** was a necessary part of the mechanism of action of these compounds. Polymerization of **7** made it impractical to test the compound directly. Dimmock and co-workers[3] have demonstrated that for the antineoplastic activity of similar compounds the intact molecule was necessary. The elimination products of their series were inactive. We feel our results (vida infra) are more consistent with the dienone **7** being the active species.

Reduction of the carbonyl of **1** ($NaBH_4$) gave compound **12**, which was essentially inactive in the mouse paralysis model (Table II). Another compound without the double bond (**13**, Table II) was also much less active than **1** in this test. Another variation carried out on the side chain was the introduction of methyl groups α to the carbonyl. Table V shows the effect of addition of two methyl groups (**27** vs. **6**) was to abolish the activity. Compound **27** cannot form a dienone. A sulfoxide (**31**), sulfone (**44** and **45**) or weakly basic nitrogen (**46**) at X gave weakly active or inactive compounds (Table III).

Table I presents the activity of a series with a wide range of aromatic substituents. Only two compounds had uninteresting activity, **22** and **26**. If one accepts the formation of **7** as the mechanism of action, then the inactivity of **22** might be due to a slowing of the rate of formation of **7** from **22**. Compound **26**, with the strongly electron-withdrawing nitro substituent, might form **7** at such a rate as to be unstable. The intermediate 4-phenyl-3-buten-2-ones (Table VII) were inactive in the mouse paralysis model.

Table IV presents the dose–response data for four of the compounds. As shown, the activity of these compounds at 1.5% was comparable to that of disodium phosphonoacetate at 5%. Compound **1** was evaluated in the hairless mouse model with HVH type II as the challenge (Table VI). Compound **1**, in this test, demonstrates antiherpes activity against HVH type II comparable to that seen against HVH type I.

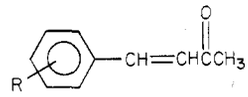
In summary, we have found a new class of antiherpes agents, readily synthesized from available starting materials. This antiherpes activity seems comparable to that of disodium phosphonoacetate in our model test systems.

Experimental Section

Melting points were determined in a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were obtained in KBr disks (solids) or as a film (liquids). NMR spectra were obtained on a Varian A-60 instrument. The compounds were assigned the *E* configuration from the NMR spectrum. Elemental analyses were determined by the Analytical Chemistry Department of Merrell Dow and were ±0.4% of the theoretical value.

Preparation of Substituted 4-Phenyl-3-buten-2-ones. Method A. (*E*)-4-[3,4-(Methylenedioxy)phenyl]-3-buten-2-one (37). Piperonal (25 g, 0.167 mol) was dissolved in acetone (200 mL). A solution of sodium hydroxide (10 g, 0.25 mol) in 250 mL of water was added in one portion, and the mixture was stirred for 1 h. The mixture was extracted with $CHCl_3$, and the organic layer was dried ($MgSO_4$) and evaporated. The residue was re-

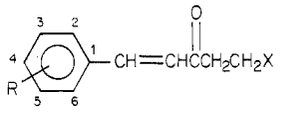
Table VII. Substituted 4-Phenyl-3-buten-2-ones



compd	R	method [a]	yield, %	mp or bp (mm), °C	ref	anal.
32	4-SCH_3	A	42	99.5-101	8	
33	4-SO_2CH_3	B	58	124-125		C, H, S
34	3,4-Cl_2	A	40	53-55	9	C, H, Cl
35	3-CF_3	C	54	90-92 (0.2)		C, H
36	2,6-Cl_2	A	72	102-103 (0.1)	9	C, H; Cl [b]
37	3,4-OCH_2O	A	63	108-109	10	C, H
38	4-Cl	A	89	47-50	11	
39	4-CN	A	23	103-106	12	C, H, N
40	4-OH	D	25	104-107	13	
41	3-OCH_3	C	61	97-105 (0.3)	14	C, H
42	4-OCH_3	C	62.5	72-72.5	15	C, H
43	3-NO_2	A	20	94.5-96	16	

[a] A = NaOH in dilute acetone–water. B = prepared by oxidation of **32** with *m*-chloroperbenzoic acid. C = cinnamic acid plus 2 equiv of methyllithium. D = 50% aqueous NaOH/acetone; see ref 12. [b] Cl: calcd, 32.97; found, 32.52.

Table VIII



compd	yield, %	mp or bp (mm) °C	anal.	ref
1	34	158-159	C, H, N, Cl	17
5		161.5-162.5	C, H, N	18
6	78	204-206	C, H, N	
9	48			
10	47	117-119	C, H	
11	15	145 (0.1)	C, H	
12	29	176-177	C, H, N	
13	40	186-187	C, H, N	
14	56	199-200	C, H, N, Cl	
15	26	195-196	C, H, Cl, S	
16	22	191-192	C, H, Cl, N, S	
17	15	192-193	C, H, N	
18	48	193-194	C, H, N, Cl	
19	37	188-189	C, H, N, Cl	19
20	29	203-204	C, H, N, Cl	
21	28	213-214.5	C, H, N, Cl	
22	17	195-196	C, H, N, Cl	
23	54	193-194	C, H, N, Cl	
24	37	180-181	C, H, N, Cl	
25	22	188-190	C, H, N, Cl	
26	39	207-208	C, H, N, Cl	
27	10	145-148	C, H, N	
28	60	146-147	C, H, N	
29	45	60-61	C, H	
30	28	95-97	C, H	
31	10	99-100	C, H	20
44	75	104-105	C, H	
45	61	145-145.5	C, H	
46	41	96-98	C, H	
47	18	liquid	C, H	
48	1	38-40	C, H	
49	27	134-135	C, H	
50	52	131-133	C, H, N	
51	82	179-181	C, H, N	
52	81	167-168	C, H, N	

crystallized from toluene to give 20 g of solid, mp 108–109 °C. Anal. ($C_{11}H_{10}O_3$) C, H.

Method C. (*E*)-4-(4-Methoxyphenyl)-3-buten-2-one (42). 4-Methoxycinnamic acid (7.12 g, 0.04 mol) was suspended in 700 mL of anhydrous ether, and the mixture was chilled to 5 °C. Methyllithium (80 mol, 46 mL of 1.8 M ether solution) was added dropwise. The mixture was stirred for 4 h at 5 °C and warmed to ambient temperature, and stirring was continued for 18 h. The mixture was poured into 500 mL of cold 1 N HCl, and the layers were separated. The ether layer was extracted with aqueous $NaHCO_3$ and brine, dried, and evaporated. The residue was recrystallized from hexane to give 4.4 g of solid, mp 72–72.5 °C. Anal. ($C_{11}H_{12}O_2$) C, H.

Mannich Reaction. 5-(4-Morpholinyl)-1-phenyl-1-penten-3-one Hydrochloride (1). A mixture of 4-phenyl-3-buten-2-one (7.3 g, 0.05 mol), paraformaldehyde (2.3 g), morpholine (4.4 g, 0.05 mol), concentrated HCl (5 mL), and ethanol (25 mL) was heated at 80 °C for 4 h. The mixture was diluted with 200 mL of acetone, chilled, and filtered. The precipitate was recrystallized from ethanol to give 4.8 g of white solid, mp 158–159 °C. Anal. ($C_{15}H_{19}NO_2$·HCl) C, H, N, Cl. The other morpholine Mannich bases were prepared following essentially this procedure. The dimethylamino compounds were prepared in ethanol with dimethylamine hydrochloride.

(*E*)-*N,N,N*-Trimethyl-3-oxo-5-phenyl-4-penten-1-aminium Iodide (6). Compound **5** (20 g, 0.084 mol) was neutralized with 300 mL of saturated $NaHCO_3$ solution, and the free base was extracted from the aqueous mixture with $CHCl_3$ (2 × 700 mL). The organic extracts were dried and evaporated. The residue was redissolved in anhydrous ether (800 mL), and methyl iodide (50 g, 0.35 mol) was added. The reaction mixture was stirred for 72 h at ambient temperature. The precipitate was filtered off, washed with anhydrous ether, and vacuum dried to give 19.1 g of solid, mp 190–192 °C. Anal. ($C_{14}H_{20}NOI$) C, H, N.

(*E*)-1-Phenyl-5-(phenylthio)-1-penten-3-one (29). 4-Phenyl-3-buten-2-one was converted to the trimethylsilyl enol ether (**8**) in THF (lithium diisopropylamide, 1.2 equiv, and trimethylsilyl chloride).[15] The Me_3Si ether so prepared had bp 92–94 °C (0.5 mm); NMR ($CDCl_3$) δ 0.2 (s, 9 H), 4.51 (s, 2 H), 6.67 (dd, 0.9, *J* = 5 and 7 Hz, 2 H), 7.27 (m, 5 H). Following the procedure of Paterson and Fleming,[5] the Me_3Si enol ether (**8**) (15 g, 0.069 mol) and chloromethyl phenyl sulfide (Aldrich Chemical Co.) (7.3 g, 0.046 mol) were dissolved in 300 mL of CH_2Cl_2 containing 0.03 g of zinc chloride. The mixture was stirred at ambient temperature for 18 h. The mixture was evaporated to dryness and the residue was purified by flash chromatography[21] (silica gel, 10% ethyl acetate in hexane). The purified material was recrystallized from hexane/acetone to give 5.6 g of solid, mp 61–62 °C. Anal. ($C_{17}H_{16}OS$) C, H.

(*E*)-Dimethyl(3-oxo-5-phenyl-4-penten-1-yl)sulfonium Iodide (10). Compound **9** (16.7 g, 0.08 mol) and methyl iodide (36.4 mL, 0.59 mol) were dissolved in 200 mL of anhydrous ether, and the solution was stirred at ambient temperature for 5 days. The precipitate was filtered off, washed with ether, and vacuum dried to give 11.9 g of cream-colored solid, mp 117–119 ° C dec. Anal. ($C_{13}H_{17}IOS$) C, H.

(*E*)-5-(Methylsulfinyl)-1-phenyl-1-penten-3-one (31) was prepared by the procedure of Leonard and Johnson.[22] Compound **9** (2.5 g, 0.012 mol) was dissolved in ethanol (20 mL), and a 0.5 M solution of $NaIO_4$ (2.7 g of $NaIO_4$, 0.127 mol) was added. The mixture was stirred for 18 h at ambient temperature, diluted with 100 mL of ethyl acetate, and filtered. The filtrate was evaporated, 250 mL of toluene was added, and the mixture was evaporated again to remove water from the residue. The residue was recrystallized from hexane/methylene chloride. A small amount of yellow precipitate was discarded. The yield was 1.2 g of a white soolid: mp 98–99 °C; IR 1690, 1610, 1460, 1370, 1095, 1040, 750, 690 cm^{-1}; NMR ($CDCl_3$) δ 2.6 (s, 3 H), 3.0–3.35 (m, 4 H), 6.72 (d, *J* = 8 Hz, 1 H), 7.2–7.8 (m, 6 H). The downfield doublet from the trans double bond was not separated from the aromatic proton multiplet. Anal. ($C_{12}H_{14}O_2S$) C, H.

(*E*)-5-(Methylsulfonyl)-1-phenyl-1-penten-3-one (44). Compound **31** (0.781 g, 3.5 mmol) was dissolved in methylene chloride (8 mL), and the solution was chilled in an ice–methanol bath. A solution of *m*-chloroperbenzoic acid (Aldrich Chemical Co.) (0.759 g, 4 mmol) in methylene chloride (4 mL) was added dropwise. After 30 min the reaction mixture was diluted to 450 mL with methylene chloride, and the solution was extracted with aqueous potassium carbonate. The organic layer was dried ($MgSO_4$) and evaporated. The solid residue was recrystallized form hexane/methylene chloride to give 0.62 g of solid: mp 104–105 °C; IR (KBr) 1680, 1660, 1620, 1300, 1260, 1140, 1100, 980, 780, 680, 520 cm^{-1}; NMR ($CDCl_3$) δ 2.9 (s, 3 H), 3.05–3.5 (m, 4 H), 6.70 (d, *J* = 6 Hz, 1 H) 7.3–7.5 (m, 5 H), 7.6 (d, *J* = 6 Hz, 1 H). Anal. ($C_{12}H_{14}O_3S$) C, H.

(*E*)-α-(2-Phenylethenyl)-4-morpholinepropanol Hydrochloride (12). The free base from the neutralization of 1.4 g (5 mmol) of **1** was dissolved in 7.5 g of ethanol. Sodium borohydride (0.1 g, 2.5 mmol) was added, and the solution was stirred at ambient temperature for 24 h. The product was precipitated by the addition of anhydrous HCl gas. The precipitate was recrystallized from ethanol/ether to give 0.36 g of white solid: mp 176–177 °C; IR (KBr) did not show a carbonyl absorption peak.

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Anal. ($C_{15}H_{21}NO_2$·HCl) C, H, N.

1-(3,4-Dichlorophenyl)-5-(4-morpholinyl)-3-pentanone Hydrochloride (13). A solution of compound 14 (0.526 g, 1.5 mmol) in ethanol (70 mL) and water (1 mL) was hydrogenated at atmospheric pressure in the presence of 0.05 g of 5% Pd/C. After 1 equiv of hydrogen had been absorbed, the catalyst was filtered off, and the filtrate was evaporated. The solid residue was recrystallized from ethanol/ether to give 0.29 g of white solid: mp 186–187 °C; NMR showed the disappearance of the vinyl protons; IR still shows carbonyl bond. Anal. ($C_{15}H_{19}Cl_2NO_2$·HCl) C, H, N.

Diethyl (*E*)-(3-Oxo-5-phenyl-4-pentenyl)phosphonate (11). A mixture of compound 5 (free base) (1.6 g, 7.9 mmol), acetic acid (0.48 g, 7.9 mmol), and triethyl phosphite (1.3 g, 7.9 mmol) was heated in a 10-mL flask equipped with a distilling head. After a period of 20 min at 125 °C, 0.42 g of distillate had been collected, and heating was stopped. Distillation of the residue gave 0.3 g of liquid: bp 170 °C (0.1 mm); ν_{max} 2990, 1680, 1670, 1610, 1450, 1240, 1090, 1050, 1025, 960, 780, 760, 680, 520 cm^{-1}; NMR ($CDCl_3$) δ 1.59 (t, J = Hz, 6 H), 1.9–2.4 (m, 2 H), 2.75–3.2 (m, 2 H), 4.12 (d of q J = 7 and 7 Hz, 4 H), 6.72 (d, J = 16 Hz, 1 H), 7.23–7.8 (m, 6 H) (includes vinyl proton). Anal. ($C_{15}H_{21}O_4P$) C, H.

Notes

Structure–Activity Relationships for Activation of Adenylate Cyclase by the Diterpene Forskolin and Its Derivatives

K. B. Seamon,*[†] J. W. Daly,[†] H. Metzger,[‡] N. J. de Souza,[§] and J. Reden[§]

Laboratory of Bioorganic Chemistry, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205, and Hoechst Pharmaceuticals Ltd., Bombay, India and Frankfurt, Germany. *Received June 28, 1982*

Forskolin (7β-acetoxy-8,13-epoxy-1α,6β,9α-trihydroxylabd-14-en-11-one), a diterpene from the Indian plant *Coleus forskohlii*, activates cyclic AMP generating systems in a number of mammalian tissues in a rapid and reversible fashion. Derivatives of forskolin have been tested for their ability to stimulate membrane adenylate cyclase from rat brain and rabbit heart, as well as cyclic AMP generation in guinea pig brain vesicular preparations, a model system for intact cells. Derivatives at the 6β- and 7β-hydroxy functions retain activity, but none have greater activity than that of forskolin. Reduction of the 11-keto function affords an active 11β-hydroxy derivative. Reduction of the 14,15-vinyl (α) substituent reduces activity, while epoxidation abolishes activity. Derivatization or lack of the 1α- and 9α-hydroxy functions results in a marked reduction in activity, emphasizing the importance of the α aspect of the molecule. However, the 1α,6β-di-*O*-acetyl derivative does retain activity. None of the inactive derivatives, which include the 14,15-epoxy, the 1,9-dideoxy, and the 1,6-diketo derivatives, antagonize the stimulatory effects of forskolin.

Forskolin is the major diterpene isolated from the roots of *Coleus forskholii*.[1] *Coleus* species have been described in ancient Hindu and Ayurvedic texts as having medicinal properties. Forskolin has positive inotropic effects on cardiac preparations.[2] These effects are related to the ability of forskolin to activate cardiac adenylate cyclase.[3,4] Forskolin also has hypotensive activity due to peripheral vasodilation.[2] This effect and its antispasmodic activity are linked to its ability to relax smooth muscle.[2] A related diterpene, coleonol, has been reported to differ from forskolin only in the configuration of the acetate group at the 7-position.[5] Coleonol displays a pharmacological profile similar to that of forskolin.[6]

Activation of adenylate cyclase by forskolin is not restricted to cardiac tissue and appears to pertain to most eukaryotic adenylate cyclases.[7–9] Forskolin appears to activate adenylate cyclase directly without the requirement of hormone receptors, guanine nucleotide regulatory proteins, or guanine nucleotides, making it a unique agent for studying the enzyme.[10] The activation is rapid and reversible. Forskolin also has the ability to activate adenylate cyclase in intact cells, resulting in increases in intracellular cyclic AMP.[7–9] We have now initiated studies on structure–activity relationships of forskolin and report here the biochemical properties of a number of forskolin derivatives. The isolation or synthesis of the forskolin derivatives have been or will be described elsewhere.[11,12]

Results

Derivatives of forskolin have been tested for their ability to stimulate adenylate cyclase in crude membranes from rat cerebral cortex. Forskolin (1) activates adenylate cyclase in this assay system about 6-fold, increasing activity

[†] NIADDK, NIH.
[‡] Hoechst Pharmaceuticals Ltd., Frankfurt, Germany.
[§] Hoechst Pharmaceuticals Ltd., Bombay, India.

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