Articles

Cardiovascular Effects of New Water-Soluble Derivatives of Forskolin

Y. Khandelwal,* K. Rajeshwari, R. Rajagopalan,* Lakshmi Swamy, A. N. Dohadwalla, N. J. de Souza, and R. H. Rupp

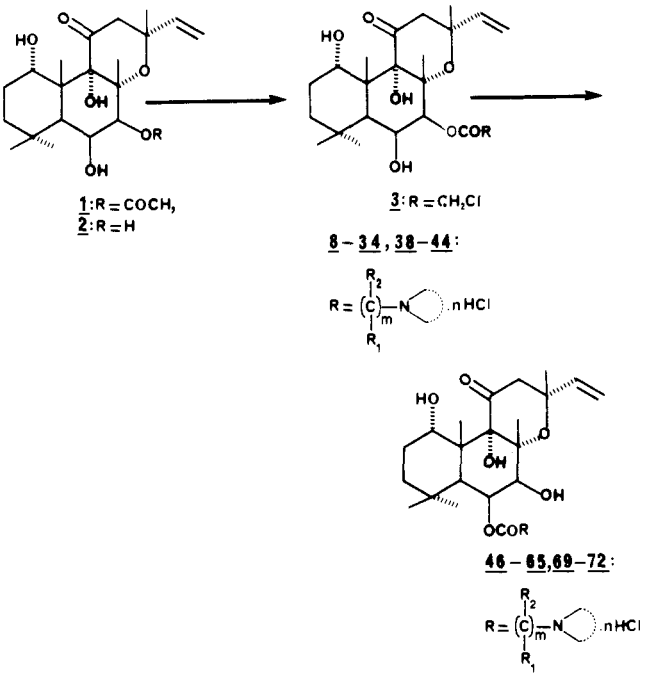
Centre for Basic Research, Hoechst India Limited, L.B.S. Marg, Mulund, Bombay 400 080, India. *Received December 21, 1987*

A series of 6- and 7-aminoacyl derivatives of 7-deacetylforskolin was prepared to provide water-soluble derivatives of the potent cardioactive diterpenoid forskolin. The compounds were evaluated for positive inotropic and blood pressure lowering properties in pharmacological models. Several derivatives displayed potent positive inotropic activity in guinea pig atria (EC_{50} = 0.16–3.0 μg/mL). In the most active compounds, the amino moiety of the aminoacyl chain corresponded to a cyclic amine, and the acyl moiety to a C_2–C_4 alkanoyl group. In vivo biological evaluation led to the selection of 6-(piperidinoacetyl)-7-deacetylforskolin hydrochloride (**49**) as a candidate for clinical development.

Forskolin (**1**), a cardioactive diterpenoid isolated from *Coleus forskohlii*, emerged out of our screening program directed toward the discovery of new leads from Indian medicinal plants.[1] Pharmacologically, the compound is characterized by potent positive inotropic activity and blood pressure lowering property in normotensive and hypertensive laboratory animals,[2] particularly when administered through the intravenous route. Its potent positive inotropic activity and vasodilatory effect has been attributed to its direct stimulation of the catalytic unit of the adenylate cyclase system.[3] Forskolin is the prototype of derivatives displaying activity by this mode of action and has consequently attracted considerable attention as a biochemical tool to study the regulation of the enzyme. In clinical studies, it has shown promising results in the treatment of diseases such as glaucoma, congestive cardiomyopathy, and asthma.[4] The poor solubility of forskolin in physiologically acceptable media, like buffers or saline, was a limiting factor in biological studies. To overcome this difficulty a program was undertaken to prepare water-soluble derivatives of forskolin that would continue to display biological properties.

In this paper we describe the synthesis of water-soluble aminoacyl derivatives of 7-deacetylforskolin (**2**) and their cardiovascular properties.

Scheme I



Chemistry

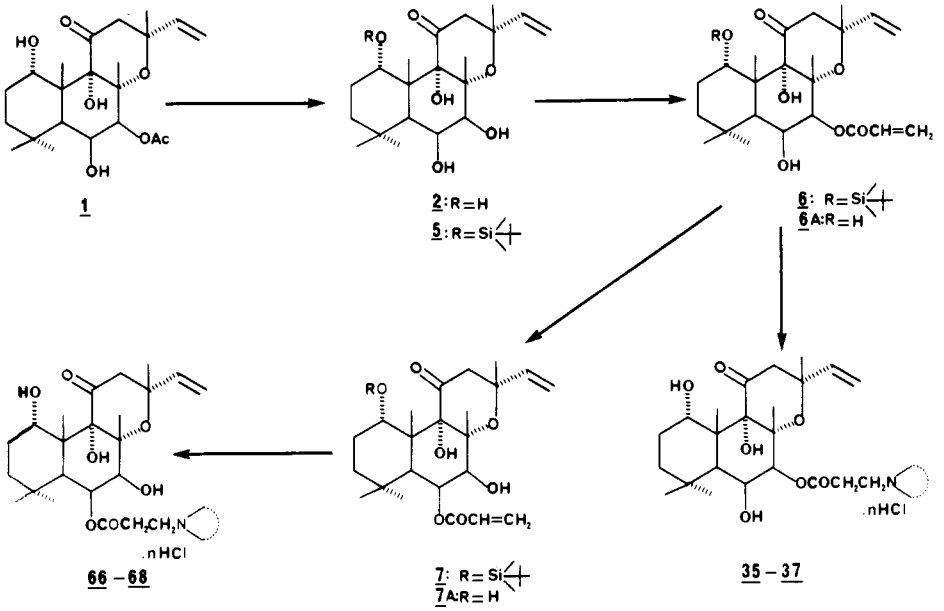
Treatment of 7-deacetylforskolin[5] with haloacyl halide in pyridine at 0–28 °C gave 7-(haloacyl)-7-deacetylforskolin selectively (Scheme I). At a higher temperature the reaction led to a mixture of 1-(haloacyl)-7-deacetyl and 7-(haloacyl)-7-deacetylforskolin. The halogen atom in 7-(haloacyl)-7-deacetylforskolin was displaced by a variety of primary and secondary amines under the usual reaction conditions to give the corresponding 7-(aminoacyl)-7-deacetylforskolin derivatives (**8–34, 38–44**).[6] Base-catalyzed migration of the 7-acyl substituent in the free base of compounds **11–23, 27–32, 39–41**, and **43** gave the corresponding 6-(aminoacyl)-7-deacetylforskolin derivatives **46–65**, **56**, and **67–72**,[6] respectively. When 7-(chloroacetyl)-7-deacetylforskolin (**3**) was treated with benzylamine, besides the normal substitution product **45**,[6] the dimeric compound **73** could be isolated. In the synthesis of 7-(3′-aminopropionyl)-7-deacetylforskolin derivatives (**35–37**)[6] and 6-(3′-aminopropionyl)-7-deacetylforskolin derivatives (**66–68**),[6] unexpected difficulties were faced. These were, therefore, synthesized by a Michael-type addition of piperidine, morpholine, and *N*-methylpiperazine on 7-acryloyl-7-deacetylforskolin (**6A**) and 6-acryloyl-7-deacetylforskolin (**7A**), respectively. The former com-

(1) Bhat, S. V.; Bajwa, B. S.; Dornauer, H.; de Souza, N. J. *Tetrahedron Lett.* **1977**, 1669.
(2) Bhat, S. V.; Dohadwalla, A. N.; Bajwa, B. S.; Dadkar, N. K.; Dornauer, H.; de Souza, N. J. *J. Med. Chem.* **1983**, *26*, 486.
(3) Seamon, K. B.; Daly, J. W.; Metzger, H.; de Souza, N. J.; Reden, J. *J. Med. Chem.* **1983**, *26*, 436.
(4) *Forskolin–Its Chemical, Biological and Medical Potential*; de Souza, N. J., Dohadwalla, A. N., Rupp, R. H., Eds.; Hechst India Limited: Bombay, 1986.
(5) Bhat, S. V.; Bajwa, B. S.; Dornauer, H.; de Souza, N. J. *J. Chem. Soc., Perkin Trans. 1* **1982**, 767.

(6) All the compounds were subjected to a process to obtain their corresponding hydrochloride salts as described in the Experimental Section. Table I indicates the compounds that formed hydrochlorides.
(7) Campbell, D. G. S.; Richter, W. *Acta Pharmacol. Toxicol.* **1967**, *25*, 345.

0022-2623/88/1831-1872$01.50/0

Scheme II



pound (**6A**) was prepared by condensation of acrylic acid with 7-deacetylforskolin (**2**). The latter compound (**7A**) was obtained by condensation of acrylic acid with 1-(*tert*-butyldimethylsilyl)-7-deacetylforskolin (**5**) and deprotection of the 1-silyloxy moiety by tetrabutylammonium fluoride, with simultaneous migration of the acryloyl group from the 7- to the 6-position (Scheme II).

Pharmacology

The blood pressure lowering activity of forskolin derivatives was assessed by intravenous administration to anesthetized normotensive cats. The compounds that exhibited EC_{20} values at doses of 1 mg/kg or less, 10 min after administration, were evaluated further for antihypertensive activity by oral administration to spontaneously hypertensive (SH) rats. The procedure for compound administration and blood pressure recordings were similar to those described in our previous paper,[2] details of which are found in the Experimental Section.

The positive inotropic activity of forskolin derivatives was tested in spontaneously beating isolated guinea pig atrial preparation.[2] The concentration at which the compounds augmented the force of contraction by 50% (EC_{50}) was determined. The compounds that exhibited good positive inotropic activity (EC_{50} < 2 μg/mL) and were soluble in water were subjected to extensive investigation of their cardiovascular activity in anesthetized dog.[3]

Results and Discussion

Aqueous Solubility. Forty-six of the 66 aminoacyl derivatives of forskolin formed hydrochloride salts as listed in Table I, which were soluble in water at concentrations of 10–75 mg/mL at ambient temperatures of 26–28 °C. Compound **20** was the only hydrochloride that was poorly soluble in water (<1 mg/mL). The aminoacyl derivatives that did not form hydrochlorides were solubilized for biological testing in propylene glycol.

Blood Pressure Lowering Activity. Of the 46 water-soluble (aminoacyl)forskolin derivatives investigated in the anesthetized cat (Table I), only 20 compounds had ED_{20} values ≤ 2 mg/kg. The most potent compounds (**22**, **41**, **39**) with ED_{20} values of 0.9, 0.8, and 0.3 mg/kg, respectively, were further tested for oral antihypertensive activity in conscious spontaneously hypertensive rat model but were found to be devoid of it. Thus, water-soluble salts of forskolin derivatives bearing 6- or 7-aminoacyl moieties as described in this study were not better than forskolin in lowering blood pressure. Poor activity was also displayed by the non-water-soluble derivatives listed in Table I, which were tested in the anesthetized cat in the same vehicle as that used for forskolin. From this latter group, the most potent compounds were weaker than forskolin by 2–3 orders of magnitude.

Positive Inotropic Activity. Five water-soluble 7-aminoacyl derivatives (compounds **13**, **21**, **31**, **34**, and **35**) and 10 water-soluble 6-aminoacyl derivatives (compounds **48–51**, **56–58**, **66**, **68**, and **71**) exhibited potent positive inotropic activity in the guinea pig atrium with EC_{50} values in the range of 0.16–3.0 μg/mL. Six water-insoluble derivatives (compounds **27**, **28**, **33**, **54**, **55**, and **60**) also displayed activity in the same region. This number of potently active compounds is too small for structure–activity relationships to be derived in this series. A few general comments can, however, be made of the effects of the two variants, namely the amino group and the alkanoyl group, on the positive inotropic activity.

With respect to variation of the amino moiety, derivatives with open chain alkylamino, arylamino, and aralkylamino residues (compounds **10**, **11**, **45–47** and **73**) did not display activity. Cyclic amino residues such as benzimidazolyl, benzotriazinyl, quinolinyl, xanthinyl, and phthalimidyl (compounds **24–28**, **33**, **38**, **42**, **60**, and **61**) did not provide water-soluble salts and are compared with forskolin as a separate group below. In all of the 14 water-soluble potent active derivatives cited above, the amino moiety was a cyclic amine such as pyrrolidine, piperidine, homopiperidine, morpholine, thiomorpholine, and *N*-methylpiperazine. These residues generally displayed a better effect when located as a 6-aminoacyl substituent than as a 7-aminoacyl substituent. Substituents in the piperidine moiety, whether of a hydrophilic nature (compounds **15**, **19**, **20**, **50**, **54**, and **55**) or of a lipophilic nature (compounds **16–18** and **51–53**) contributed neither to improvement in water solubility nor to increase in activity.

The alkanoyl portion of the aminoacyl substituent was varied in the piperidinoacyl, morpholinoacyl, and (*N*-methylpiperazino)acyl series. In the piperidinoacyl series consisting of 10 compounds (**14**, **30**, **35**, **39**, **43**, **49**, **63**, **66**, **69**, and **72**), variation in chain length of the alkanoyl moiety from acetyl to pentanoic in both the 7- and 6-substituted

Table I. 6- and 7-Aminoacyl Derivatives of 7-Deacetylforskolin[a]

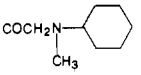
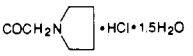
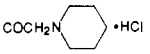
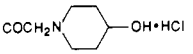
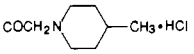
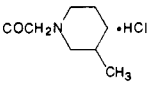
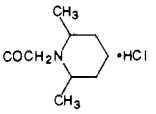
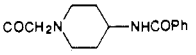
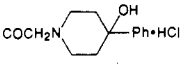
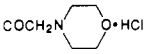
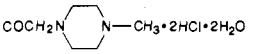
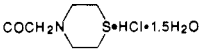
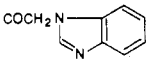
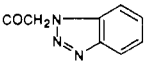
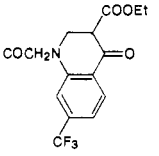
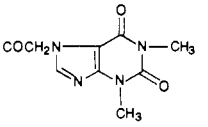
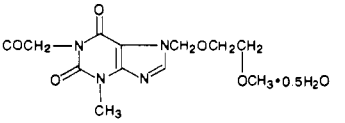
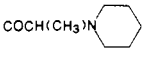
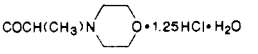
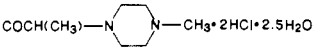
no.	R_6	R_7	mp, °C	formula[b]	EC_{50} (GP atrium), μg/mL	ED_{20} (cat BP), mg/kg iv
8	H	$COCH_2NHBOC$	184–186	$C_{27}H_{43}NO_9$	9.2	NA
9	H	$COCH_2NHPh$	186–190	$C_{28}H_{39}NO_7$	>10	2
10	H	$COCH_2NHC(CH_3)_3 \cdot HCl \cdot H_2O$	195 dec	$C_{26}H_{43}NO_7 \cdot HCl \cdot H_2O$	>30	1
11	H	$COCH_2NEt_2 \cdot HCl \cdot H_2O$	170–172	$C_{26}H_{43}NO_7 \cdot HCl \cdot H_2O$	10	2
12	H		198–200	$C_{29}H_{47}NO_7$	NA	10
13	H		189–192	$C_{26}H_{41}NO_7 \cdot HCl \cdot 1.5H_2O$	1.8	4
14	H		194–197	$C_{27}H_{43}NO_7 \cdot HCl$	>30	10
15	H		185–190 dec	$C_{27}H_{43}NO_8 \cdot HCl$	>30	3
16	H		196–199	$C_{28}H_{45}NO_7 \cdot HCl$	>30	10
17	H		247–249	$C_{28}H_{45}NO_7 \cdot HCl$	>30	10
18	H		232–234	$C_{29}H_{47}NO_7 \cdot HCl$	NA	NA
19	H		210–212	$C_{34}H_{50}N_2O_9$	NA	3
20	H		119–121	$C_{33}H_{47}NO_8 \cdot HCl$	NA	NA
21	H		174–178	$C_{26}H_{41}NO_8 \cdot HCl$	0.35	ND
22	H		193–194	$C_{27}H_{44}N_2O_7 \cdot 2HCl \cdot 2H_2O$	>30	0.9
23	H		169–171	$C_{26}H_{41}NO_7S \cdot HCl \cdot 1.5H_2O$	>30	3
24	H		247	$C_{29}H_{38}N_2O_7$	NA	10
25	H		183–185	$C_{28}H_{37}N_3O_7$	>30	3
26	H		178–180	$C_{35}H_{42}F_3NO_{10}$	NA	ND
27	H		198	$C_{29}H_{40}N_4O_9 \cdot H_2O$	0.74	3
28	H		176–180	$C_{32}H_{46}N_4O_{11} \cdot 0.5H_2O$	2	6
29	H	$COCH(CH_3)NHPh$	168–169	$C_{29}H_{41}NO_7$	NA	3
30	H		199–201	$C_{28}H_{45}NO_7$	>30	4.5
31	H		185–187	$C_{27}H_{43}NO_8 \cdot 1.25HCl \cdot H_2O$	0.27	2
32	H		221–223	$C_{28}H_{46}N_2O_7 \cdot 2HCl \cdot 2.5H_2O$	NA	10

Table I (Continued)

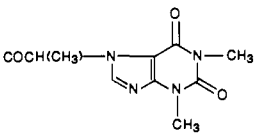
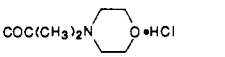
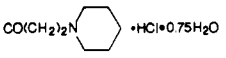
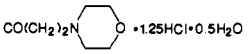
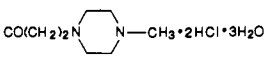
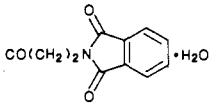
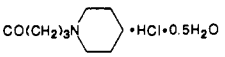
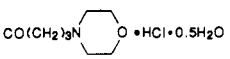
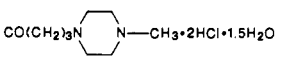
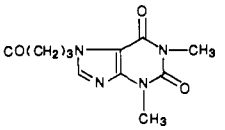
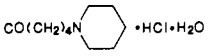
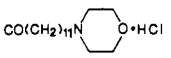
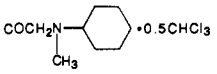
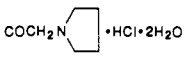
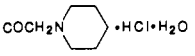
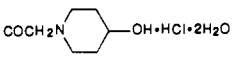
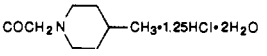
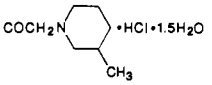
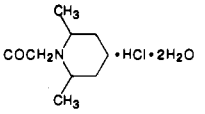
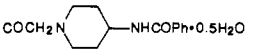
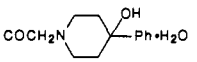
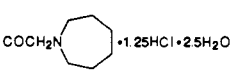
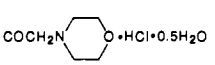
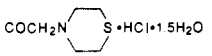
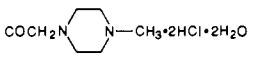
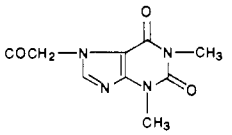
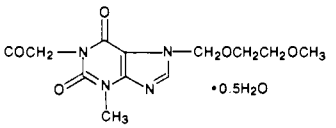
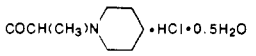
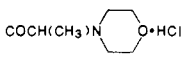
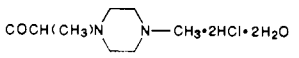
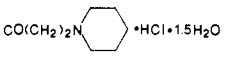
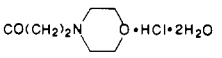
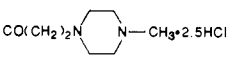
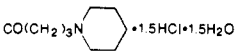
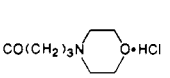
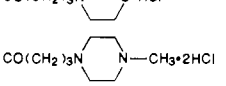
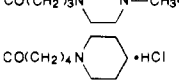
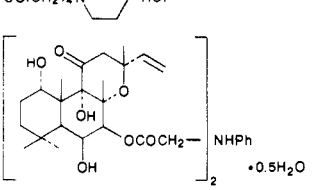
no.	R_6	R_7	mp, °C	formula[b]	EC_{50} (GP atrium), μg/mL	ED_{20} (cat BP), mg/kg iv
33	H		264–265	$C_{30}H_{42}N_4O_9$	0.9	5
34	H		274	$C_{28}H_{45}NO_8 \cdot HCl$	3	1.8
35	H		225–227	$C_{28}H_{45}NO_7 \cdot HCl \cdot 0.75H_2O$	1	ND
36	H		206–209	$C_{27}H_{43}NO_8 \cdot 1.25HCl \cdot 0.5H_2O$	NA	ND
37	H		186–188	$C_{28}H_{46}N_2O_7 \cdot 2HCl \cdot 3H_2O$	NA	ND
38	H		245–247	$C_{31}H_{39}NO_9 \cdot H_2O$	>30	10
39	H		178–181	$C_{29}H_{47}NO_7 \cdot HCl \cdot 0.5H_2O$	>30	0.3
40	H		203–208	$C_{28}H_{45}NO_8 \cdot HCl \cdot 0.5H_2O$	10	1.8
41	H		203–207	$C_{29}H_{48}N_2O_7 \cdot 2HCl \cdot 1.5H_2O$	NA	0.8
42	H		148–150	$C_{31}H_{44}N_4O_9$	NA	3
43	H		223–224	$C_{30}H_{50}NO_7 \cdot HCl \cdot H_2O$	30	1
44	H		134–140	$C_{36}H_{61}NO_8 \cdot HCl$[c]	NA	1.6
45	$COCH_2NHCH_2Ph \cdot HCl \cdot H_2O$	H	169–172	$C_{29}H_{41}NO_7 \cdot HCl \cdot H_2O$	10	3.5
46	$COCH_2NEt_2 \cdot HCl \cdot 2H_2O$	H	160–163	$C_{28}H_{43}NO_7 \cdot HCl \cdot 2H_2O$	NA	NA
47		H	137–139	$C_{29}H_{47}NO_7 \cdot 0.5CHCl_3$[d]	NA	3.5
48		H	137–139	$C_{26}H_{41}NO_7 \cdot HCl \cdot 2H_2O$	3.0	1.0
49		H	204–205	$C_{27}H_{43}NO_7 \cdot HCl \cdot H_2O$	1.8 (6)	3.0
50		H	187–190	$C_{27}H_{43}NO_8 \cdot HCl \cdot 2H_2O$	3.0	NA
51		H	125–128	$C_{28}H_{45}NO_7 \cdot 1.25HCl \cdot 2H_2O$	2.6	NA
52		H	188–190	$C_{28}H_{45}NO_7 \cdot HCl \cdot 1.5H_2O$	>30	5.4
53		H	171–173	$C_{29}H_{47}NO_7 \cdot HCl \cdot 2H_2O$	NA	NA
54		H	122–124	$C_{34}H_{48}N_2O_8 \cdot 0.5H_2O$	1.2	4.0
55		H	123–125	$C_{33}H_{47}NO_8 \cdot H_2O$	3.0	5.0
56		H	192	$C_{28}H_{39}NO_7 \cdot 1.25HCl \cdot 2.5H_2O$	1.9	1.0
57		H	152–155	$C_{26}H_{41}NO_8 \cdot HCl \cdot 0.5H_2O$	0.5 (6)	3.0

Table I (Continued)

no.	R_6	R_7	mp, °C	formula[b]	EC_{50} (GP atrium), μg/mL	ED_{20} (cat BP), mg/kg iv
58		H	173–176	$C_{26}H_{41}NO_7S\cdot HCl\cdot 1.5H_2O$	1.5	5.0
59		H	182–184	$C_{27}H_{44}N_2O_7\cdot 2HCl\cdot 2H_2O$	30	1.3
60		H	149–153 dec	$C_{29}H_{40}N_4O_9$	3.0	4.5
61		H	158–162	$C_{32}H_{46}N_4O_{11}\cdot 0.5H_2O$	NA	NA
62	$COCH(CH_3)NHPh$	H	218–219	$C_{29}H_{41}NO_7$	NA	6.2
63		H	232–235	$C_{28}H_{45}NO_7\cdot HCl\cdot 0.5H_2O$	NA	1
64		H	179–180	$C_{27}H_{43}NO_8\cdot HCl$[e]	>30	3
65		H	185–189	$C_{28}H_{46}N_2O_7\cdot 2HCl\cdot 2H_2O$	NA	3
66		H	195–197	$C_{28}H_{45}NO_7\cdot HCl\cdot 1.5H_2O$[f]	0.5	1
67		H	165–167	$C_{27}H_{43}NO_8HCl\cdot 2H_2O$	NA	NA
68		H	221–222	$C_{28}H_{46}N_2O_7\cdot 2.5HCl$	0.2	1.4
69		H	162–164	$C_{29}H_{47}NO_7\cdot 1.5HCl\cdot 1.5H_2O$[g]	NA	3
70		H	232–233	$C_{28}H_{45}NO_8\cdot HCl$	NA	2
71		H	249–250	$C_{29}H_{48}N_2O_7\cdot 2HCl$	0.2 (4)	1
72		H	245–247	$C_{30}H_{49}NO_7\cdot HCl$	>30	1
73			127–130	$C_{51}H_{73}NO_{14}\cdot 0.5H_2O$	NA	NA
forskolin					0.02 (6)	0.038
dobutamine					0.3 (4)	
minoxidil						0.9

[a] NA = not active; ND = not done; EC_{50} and ED_{20} values were determined graphically from dose–response curves obtained by measuring the positive inotropic and blood pressure lowering activity, respectively, at different concentrations of the compounds in triplicate. Number of observations exceeding three are mentioned in parentheses. [b] The elemental analysis (C, H, N, and Cl) for all new compounds were within ±0.4% of the theoretical values. [c] C: calcd, 64.33; found, 63.72. [d] Cl: calcd, 9.17; found, 10.46. [e] C: calcd, 59.40; found, 58.78. [f] C: calcd, 58.90; found, 58.45. [g] Cl: calcd, 8.83; found, 8.12.

series showed that the most potent compounds (**35**, **66**) were those with a propionyl group in the piperidinoacyl chain. In the 6-piperidinoacyl series, the increase in the chain length from acetyl to propionyl provided compound **66**, which was about 3.5 times more potent than compound **49**. The branched 2-piperidinopropionyl chain (compound **63**) contributed to a loss of activity in comparison to the straight-chained 3-piperidinopropionyl derivative **66**. In the morpholinoacyl series, of the 10 compounds (**21**, **31**, **34**, **36**, **40**, **44**, **57**, **64**, **67**, and **70**) investigated, the acetyl group provided potent compounds (**21**, **57**) in both the 6- and 7-series. Increasing the chain length to the 2-propionyl group led to as active a compound (**31**) in the 7-series, but to loss of activity in the 6-series (compound **64**). Further increase in the chain length, whether in straight or branched chains, led to loss of activity. In the (*N*-methylpiperazino)acyl series, of the eight compounds (**22**, **32**, **37**, **41**, **59**, **65**, **68**, and **71**) investigated, activity was surprisingly observed only for two compounds (**68** and **71**) with the propionyl and butyryl chains as 6-substituents. Compounds **68** and **71** were the most potent positive inotropic compounds of the entire series in the isolated guinea pig atria model.

Of the water-insoluble (aminoacyl)forskolin derivatives, only two xanthinyl derivatives (compounds **27** and **33**) displayed weak positive inotropic activity with EC_{50} values equal to 0.74 and 0.9 μg/mL, respectively, in comparison to that of forskolin (0.02 μg/mL).

In Vivo Studies. The water-soluble derivatives (**13**, **21**, **31**, **35**, **49**, **57**, **58**, **66**, **68**, and **71**), which displayed positive

Table II. Effect of Forskolin Derivatives on Left Ventricular dP/dt max in Anesthetized Dogs and Acute Toxicity (LD_{50}) Determination in Mice[a]

compd no.	iv dose, mg/kg, to product 50% increase in Lv dP/dt max	LD_{50} in mice, mg/kg ip
21	0.035 (3)	25
31	0.04 (5)	30
49	0.052 (5)	85
57	0.11 (3)	82.5
66	0.3 (1)	30
71	0.41 (1)	65
forskolin (1)	0.007 (5)	92

[a] ED_{50} was calculated from the dose–response curve. LD_{50} value was determined according to Campbell and Richter (1967).[7] Number in parentheses indicates number of observations.

Table III. Cardiovascular Profile of 6-(Piperidinoacetyl)-7-deacetylforskolin (**49**) in Anesthetized Dogs[a]

dose, mg/kg iv	percent increase: Lv dP/dt max	percent increase: heart rate	fall in mean BP, mmHg
0.03	48 ± 23.5	6.2 ± 0.7	
0.1	64 ± 17[c]	8.8 ± 3[b]	8.8 ± 8
0.3	94 ± 19[d]	9.8 ± 2[d]	30.2 ± 6[e]
1	123 ± 26[d]	13.8 ± 4[b]	72 ± 8[e]

[a] Values are maximum response from control (±SEM). [b] $p < 0.05$. [c] $p < 0.02$. [d] $p < 0.01$. [e] $p < 0.001$ (compared to control values). Number of observations is five.

inotropic activity with ED_{50} values ≤ 2 μg/mL in the in vitro guinea pig model were subjected to extensive in vivo cardiovascular hemodynamic studies in the anesthetized dog. The acute toxicity in mice was also studied. In the anesthetized dog, the left ventricular dP/dt max values (Table II) were clearly indicative of the potent positive inotropic properties of the compounds. The duration of the positive inotropic activity, the effects of the compounds on heart rate and blood pressure, and the acute toxicity of the compounds provided a basis for selection of a compound in this series to be considered as a candidate for clinical development. 6β-(piperidinoacetyl)-7-deacetylforskolin (**49**, Tables II and III) was assessed to be such a candidate from this series.

Conclusion

Alteration of the forskolin molecule at the 6- and 7-positions, based on our proposed model of forskolin action in which it is suggested that the hydroxy and acetoxy functions at the 6- and 7-positions, respectively, are not critical for activity,[8] has led to a novel series of potent positive inotropic agents. The water solubility of these second-generation forskolin analogues removes a handicap with which forskolin is encumbered in its clinical development as a positive inotropic drug.

Experimental Section

Chemistry. Melting points were determined with a Kofler hot stage apparatus and are uncorrected. IR spectra were determined with a Perkin-Elmer 157 spectrophotometer for KBr disks. ^1H NMR spectra were measured for solutions in $CDCl_3$ with a JEOL FT-90 spectrometer unless mentioned otherwise (Me_4Si as internal standard). Usual workup refers to diluting with water, followed by extracting with ethyl acetate or chloroform, washing the organic layer with water, drying over Na_2SO_4 and evaporating in vacuo. Petroleum ether refers to the fractions of bp 60–80 °C. Precoated (silica gel $60F_{254}$) TLC plates were used to examine the purity of compounds. Visualization was done by spraying with anisaldehyde/H_2SO_4, reagent and heating the plate at 110 °C. All compounds were homogeneous by TLC analysis and exhibited proper spectral characteristics. Microanalytical results on derivatives were within ±0.4% of theoretical values. The general procedures for preparation of the derivatives are illustrated by the following examples.

7β-Acetoxy-1α-[(*tert*-butyldimethylsilyl)oxy]-6β,9α-dihydroxy-8,13-epoxylabd-14-en-11-one (4). *tert*-Butylchlorodimethylsilane (3.6 g 23.88 mmol) was added to a mixture of imidazole (2.52 g, 37 mmol), **1** (6.0 g 14.63 mmol), and anhydrous dimethylformamide (12 mL), and the mixture was stirred for 22 h at 70 °C and concentrated in vacuo. The usual workup gave **4**, which was purified by flash chromatography over silica gel with ethyl acetate/hexane (3:7) as eluent, gave **4**: mp 137–139 °C; yield, 80%.

1α-[(*tert*-Butyldimethylsilyl)oxy]-8,13-epoxy-6β,7β,9α-trihydroxylabd-14-en-11-one (5). Potassium carbonate (2.4 g, 17.39 mmol) was added to a solution of **4** (0.4 g, 0.76 mmol) in a mixture of methanol (34 mL) and water (12 mL), and the mixture was stirred for 24 h at room temperature and then concentrated in vacuo. The usual workup gave **5**, which was purified by flash chromatography with chloroform/diisopropyl ether (55:45) as eluent, gave **5**: mp 60–62 °C; yield, 90%.

7β-(Chloroacetoxy)-8,13-epoxy-1α,6β,9α-trihydroxylabd-14-en-11-one (3). A solution of chloroacetyl chloride (0.35 mL) in dichloromethane (2 mL) was added to a mixture of **2** (1.5 g, 4.08 mmol) dissolved in dichloromethane (10 mL) and dry pyridine (0.74 mL) cooled in an ice bath. The reaction mixture was stirred for half an hour at 0 °C and concentrated in vacuo. The residue was extracted with dichloromethane. The organic layer was separated, washed with dilute HCl (10%), saturated sodium bicarbonate solutions, water and brine sequentially, and finally dried over anhydrous sodium sulfate and concentrated. The residue was purified by flash chromatography with ethyl acetate/pertroleum ether (1:3) as eluent. The compound isolated from the column was crystallized from ethyl acetate/petroleum ether: mp 200–202 °C; yield, 90%.

8,13-Epoxy-7β-(piperidinoacetoxy)-1α,6β,9α-trihydroxylabd-14-en-11-one Hydrochloride (14). A mixture of piperidine (50 mL) and **3** (4 g) was stirred at 50–60 °C for 1.5 h. The reaction mixture was concentrated in vacuo. Usual workup gave the product, which was purified by MPLC with ethyl acetate/petroleum ether/triethylamine (44:66:0.5) as eluent. The compound was crystallized from chloroform/petroleum ether: mp 162–164 °C; yield, 80%. It was converted into its hydrochloride by following the general procedure. ^1H NMR ($CDCl_3$ + 1 drop DMSO-d_6) δ 1.02, 1.28, 1.32, 1.42, 1.74 (s, $5CH_3$), 2.36 (d, J_{gem} = 16.2 Hz, 12-C*H*) 3.16 (d, J_{gem} = 16.2 Hz, 12-C*H*) 4.12 (s, $COCH_2$) 4.4 (br s, 1β-C*H*, 6α-C*H*) 4.82 (dd, J_{cis} = 10.8 Hz, J_{gem} = 2 Hz, vinylic *H*) 5.06 (dd, J_{trans} = 18 Hz, J_{gem} = 2 Hz, vinylic *H*) 5.4 (d, $J_{6,7}$ = 4.5 Hz, 7α-CH) 6.0 (dd, J_{cis} = 10.8 Hz, J_{trans} = 18 Hz, vinylic *H*); IR (KBr) 3320, 2950, 1740, 1715, 1260, 1220, 1120, 1060 cm^{-1}.

Similarly, compounds **9–23**, **29–32**, **34**, **39–41**, **43**, and **44** were prepared from the corresponding 7-[(haloacyl)oxy]-8,13-epoxy-1α,6β,9α-trihydroxylabd-14-en-11-one.

8,13-Epoxy-6β-(piperidinoacetoxy)-1α,7β,9α-trihydroxylabd-14-en-11-one Hydrochloride Hydrate (49). Method 1. The free base of **14** (0.1 mol) was stirred with sodium methoxide (0.1 mol) in dry dioxane (25 mL) for 12 h. The reaction mixture was then concentrated in vacuo at 0 °C, and the residue was extracted with dichloromethane. The extract was washed with water and dried over anhydrous sodium sulfate. The extract on concentration gave the residue, which on purification by MPLC gave 8,13-epoxy-6β-(piperidinoacetoxy)-1α,7β,9α-trihydroxylabd-14-en-11-one: mp 111–113 °C; yield, 70%.

Method 2. The free base of **14** (1.0 g) was refluxed in dry piperidine (10 mL) for 12 h. The reaction mixture was concentrated in vacuo, and the usual workup gave a product, which was purified by flash chromatography and yielded 8,13-epoxy-6β-(piperidinoacetoxy)-1α,7β,9α-trihydroxylabd-14-en-11-one: mp 111–113 °C; yield, 85%. Compound isolated by either method was converted to its hydrochloride by following the general procedure: ^1H NMR ($CDCl_3$) δ 0.96, 1.08, 1.32, 1.4, 1.52 (s, $5CH_3$), 2.4 (d, $J_{5,6}$ = 3.5 Hz, 5-C*H*) 2.46 (d, J_{gem} = 18 Hz, 12-C*H*) 3.2 (d, J_{gem} = 18 Hz, 12-C*H*) 3.4 (m, N-CH_2) 3.84 (s, $COCH_2$) 4.3 (d, $J_{6,7}$ = 4.5 Hz, 7-C*H*) 4.62 (m, 1-C*H*) 4.94 (dd, J_{cis} = 10.8 Hz, J_{gem} =

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2 Hz, vinylic *H*) 5.1 (dd, J_{trans} = 18 Hz, J_{gem} = 2 Hz, vinylic *H*) 5.9 (dd, $J_{6,7}$ = 4.5 Hz, $J_{5,6}$ = 3.5 Hz, 6-C*H*) 6.08 (dd, J_{trans} = 18 Hz, J_{cis} = 10.8 Hz, vinylic *H*); IR (KBr) 3350, 2840, 1770, 1720, 1460, 1420, 1230, 1200, 1100, 1055 cm^{-1}.

Similarly, compounds **46–65** and **69–72** were prepared from corresponding 7-aminoacyl derivatives.

8,13-Epoxy-7β-(theophyllinoacetoxy)-1α,6β,9α-trihydroxylabd-14-en-11-one Monohydrate (27). Theophylline (0.198 g, 1.1 mmol) in anhydrous dimethylformamide (8 mL) was added dropwise to a stirred mixture of potassium carbonate (0.152 g, 1.1 mmol) and anhydrous dimethylformamide (7 mL). After the addition, stirring was continued for an additional 2 h. Compound **3** (0.445 g, 1.0 mmol) in anhydrous dimethylformamide (4 mL) was then added to the reaction mixture, and the resultant mixture was stirred overnight at room temperature.

The reaction mixture was poured on ice and extracted with ethyl acetate. The extract was washed with water and brine and dried over anhydrous sodium sulfate. The residue obtained from concentration of the extract, on purification by flash chromatography with ethyl acetate/diisopropyl ether mixture as eluent, gave **27**: mp 198 °C; yield, 65%.

Similarly, compounds **24**, **28**, **33**, and **42** were prepared from corresponding 7-(haloacyl)-8,13-epoxy-1α,6β,9α-trihydroxylabd-14-en-11-ones.

7β-(Acryloyloxy)-1α-[(*tert*-butyldimethylsilyl)oxy]-6β,9α-dihydroxy-8,13-epoxylabd-14-en-11-one (6). Acrylic acid (2.28 mL, 33.2 mmol) was added to a mixture of DCC (16.56 g, 80.38 mmol) and 4-(dimethylamino)pyridine (2.02 g, 16.56 mmol) in ethyl acetate (150 mL) g, stirred for 10 min at room temperature; **5** (8.0 g, 16.1 mmol) was added to the above reaction mixture, and stirring was continued overnight. The excess of DCC in the reaction mixture was decomposed by adding acetic acid, and the reaction mixture was filtered. The filtrate was washed with saturated sodium bicarbonate solution, water, and brine. The organic layer was dried over anhydrous sodium sulfate and concentrated. The residue was purified by flash chromatography with ethyl acetate/petroleum ether (1:3) as eluant, yield 40%. The compound obtained by column chromatography was used without crystallization for the next step.

Similarly, compound **6A** was prepared from **2**; compounds **8** and **38** were prepared by condensation of **2** with the corresponding amino acids.

6β-(Acryloyloxy)-1α-[(*tert*-butyldimethylsilyl)oxy]-7β,9α-dihydroxy-8,13-epoxylabd-14-en-11-one (7). Sodium hydroxide (1 N, 8.4 mL) was added to a stirred solution of **6** (2.4 mg) in acetonitrile/water (2:1, 120 mL) at room temperature. Stirring was continued for 25 min. The reaction mixture was concentrated in vacuo and extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous sodium sulfate and concentrated. the residue was purified by flash chromatography with ethyl acetate/petroleum ether (9:41) as eluant: mp 164–166 °C; yield, 65%.

6β-(Acryloyloxy)-8,13-epoxy-1α,7β,9α-trihydroxylabd-14-en-11-one (7A). Tetrabutylammonium fluoride trihydrate (2.8 g, 8.87 mmol) was added to a stirred solution of **6** (4.0 g, 7.46 mmol) in dry THF (10 mL). The reaction mixture was stirred for 30 min at room temperature. The usual workup gave **7A**, which was purified by flash chromatography with acetonitrile/chloroform (1:9) as eluant, crystallized from chloroform/petroleum ether: mp 206–208 °C; yield, 85%.

8,13-Epoxy-6β-[(3′-piperidinopropionyl)oxy]-1α,7β,9α-trihydroxylabd-14-en-11-one Hydrochloride Sesquihydrate (66). Method 1. Dry piperidine (1 mL) was added to a stirred solution of **7** (0.5 g, 0.93 mmol) in dry ether (10 mL). Stirring was continued for an additional 1 h at room temperature. The reaction mixture was concentrated under high vacuum and extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous sodium sulfate and concentrated. The residue was dissolved in dry THF (20 mL), and tetrabutylammonium fluoride (1 M solution in THF, 1.0 mL, 1.0 mmol) was added. The reaction mixture was stirred for half an hour at room temperature. The usual workup gave the product, which was purified by flash chromatography with ethyl acetate containing 1% Et_3N as eluant. The product isolated from the column was crystallized with benzene/*n*-pentane: mp 149–50 °C; yield, ca. 50%. It was converted to its hydrochloride by following the general procedure.

Method 2. Dry piperidine (1 mL) was added to a stirred solution of **7A** (0.225 g, 0.53 mmol) in dry dichloromethane (35 mL). The reaction mixture was stirred for 16 h. The usual workup gave the product, which was purified by flash chromatography with ethyl acetate/petroleum ether/triethylamine (55:45:1) as eluant, crystallized with ethyl acetate/petroleum ether: mp 188–90 °C; yield, 63%. It was converted to its hydrochloride by following the general procedure. Similarly, compounds **67** and **68** were prepared.

General Method for the Preparation of Hydrochloride Salts. Diethyl ether (10 mL saturated with dry HCl gas at 0 °C) was added to a methanolic solution of 8,13-epoxy-7β-(piperidinoacetoxy)-1α,6β,9α-trihydroxylabd-14-en-11-one (0.5 mg in 5 mL of methanol). The mixture was diluted with an excess of dry diethyl ether, and the precipitated solid was separated by filtration. Recrystallization of the solid from methanol/diethyl ether gave **14**.

Positive Inotropic Activity. Guinea Pig Atrium. Positive inotropic activity was assessed with spontaneously beating guinea pig atria. The atria of the freshly sacrificed guinea pig were isolated and suspended in Ringer solution at 32 °C in an organ bath of 10-mL capacity. The tissue was aerated with a gaseous mixture of 95% oxygen and 5% carbon dioxide. The force of contraction was recorded on a 4 channel Nihon-Kohden recorder through an isometric strain gauge. An initial tension of 0.5–1 g was given to the preparation. Stabilization time for the preparation was 30 min. According to the solubility, forskolin derivatives were dissolved either in water or in propylene glycol (concentration 1 mg/mL). After the basal response was taken, the test compounds were administered at 0.01–30 μg/mL on a cumulative basis, and responses were recorded. A contact time of 7 min was given for each dose. EC_{50} values were calculated from dose–response curves. Forskolin was used as a standard drug; its EC_{50} value was 0.022 μg/mL.

Hypotensive Activity in Anesthetized Cat. Cats of either sex weighing 3–4 kg were anesthetized with ether anesthesia and maintained with chloralose anesthesia (70 mg/kg iv). Mean arterial pressure was recorded through a Gould electronic P23XL transducer on a 4 channel Nihon-Kohden recorder. According to their solubility, forskolin derivatives were dissolved either in distilled water or in propylene glycol (concentration 10 mg/mL) and administered intravenously through the femoral vein at various dose levels of 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 mg/kg. Ten minutes after administration of compound, the fall in mean blood pressure was recorded. ED_{20} values were calculated from the log dose–response curve. Minoxidil was used as a standard drug. Its ED_{20} value was 0.9 mg/kg i.v.

Blood Pressure Lowering Activity in the Conscious Spontaneously Hypertensive Rat Model. Male spontaneously hypertensive rats (SH) (250–300 g, 12–16 weeks old) of Okamoto strain obtained in house were used. Rats were warmed at 37–38 °C in a heating chamber for 10 min prior to blood pressure determination. Systolic blood pressure was measured in conscious rats by the tail-cuff method, utilizing a piezo electric crystal for the detection of pressure pulse, an aneroid manometer for measuring pressure, and a cardioscope (BPL, India) for visualizing the disappearance and/or appearance of the pressure pulse. The test compounds was administered in SH rats in doses of 25 mg/kg po daily once for 5 days, in a volume 1 mL/kg. One group served as a control and received vehicle. Systolic blood pressure was recorded before the first application and 2 h after each drug administration. The fall in systolic blood pressure was determined as the difference between the posttreatment blood pressure reading and the initial reading and evaluated statistically by using the paired *t* test.

Anesthetized Dog Model. Male mongrel dogs of 10–18-kg weight range were anesthetized with pentobarbital sodium 35 mg/kg iv and subsequently maintained under anesthesia with continuous infusion of pentobarbitone 3–5 mg/kg per h. Trachea was intubated for spontaneous breathing. A cannula was inserted into the femoral vein for administering test compounds. The femoral artery blood pressure was recorded through a Gould electronic P23XL transducer on 4 channel Nihon-Kohden recorder. The left ventricular pressure was recorded through a Millar catheter tip pressure transducer (Model PC 350) inserted through the left carotid artery, and the signals were differentiated

through Nihon-Kohden differentiator for recording left ventricular dP/dt max. All parameters were recorded on a strip chart recorder. The lead(II) electrocardiogram was recorded on a BPL monitor.

Compounds were dissolved in distilled water (concentration 1–10 mg/mL). Each dose of the test compound was administered in a volume of 0.1 mL/kg. According to the in vitro positive inotropic activity, doses were selected between 0.01 and 3 mg/kg. Four to six doses were selected in order to establish a dose–response relationship. A 10–30-min interval was given in between the doses. In each experiment the activity of one compound was assessed. The inotropic activity of the compound was determined by measuring the percent change in dP/dt max over the initial value. ED_{50} was calculated from the dose–response curve. Forskolin was used as a standard drug. Its ED_{50} value was 0.007 mg/kg iv.

Acute Toxicity Studies. Acute toxicity (LD_{50}) was evaluated in mice (18–20 g) of either sex. Test compounds were dissolved in distilled water or suspended in 0.5% (carboxymethyl)cellulose and injected intraperitoneally at dose levels of 10, 30, 100, 300, and 1000 mg/kg in a volume of 1 mL/100 g of body weight. The animals were observed for 48 h, and the approximate LD_{50} values were calculated according to the methods described by Campbell and Richter.[7]

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Registry No. 1, 66575-29-9; 2, 64657-20-1; 3, 111124-74-4; 4, 105559-76-0; 5, 105535-46-4; 6, 115076-57-8; 6A, 115077-01-5; 7, 115076-58-9; 7A, 115076-98-7; 8, 105559-75-9; 9, 115076-59-0; 10·HCl, 115076-60-3; 11·HCl, 105535-50-0; 12, 115116-35-3; 13·HCl, 111124-59-5; 14, 105535-79-3; 14·HCl, 105617-19-4; 15·HCl, 115076-61-4; 16·HCl, 115076-62-5; 17·HCl, 115076-63-6; 18·HCl, 115076-64-7; 19, 115076-65-8; 20·HCl, 115076-66-9; 21·HCl, 105535-53-3; 22·2HCl, 111124-58-4; 23·HCl, 115076-67-0; 24, 115076-68-1; 25, 115076-69-2; 26, 115092-13-2; 27, 115076-70-5; 28, 115092-14-3; 29, 115076-71-6; 29 (R^6 = H, R^7 = $COCH(Cl)CH_3$), 115077-04-8; 30, 111124-61-9; 31·7·25HCl, 115076-72-7; 32·2HCl, 111124-63-1; 33, 115076-73-8; 34·HCl, 115076-74-9; 34(R^6 = H, R^7 = $COCCCH_3)_2Cl$, 115077-05-9; 35·HCl, 115076-75-0; 36·7·25HCl, 115076-76-1; 37·2HCl, 115076-77-2; 38, 115076-78-3; 39·HCl, 111124-60-8; 39 (R^6 = H, R^7 = $Co(CH_2)_3Cl$), 113462-51-4; 40·HCl, 115116-36-4; 41·2HCl, 115116-37-5; 42, 115076-79-4; 43·HCl, 115116-38-6; 43 (R^6 = H, R^7 = $CO(CH_2)_4Cl$), 115077-02-6; 44·HCl, 115076-80-7; 44(R^6 = H, R^7 = $CO(CH_2)_{11}Cl$), 115077-03-7; 45·HCl, 115076-81-8; 46·HCl, 115116-39-7; 47, 111149-17-8; 48·HCl, 115116-40-0; 49·HCl, 114376-11-3; 50·HCl, 115076-82-9; 51·1.25HCl, 115076-83-0; 52·HCl, 115076-84-1; 53·HCl, 115076-85-2; 54, 115076-86-3; 55, 115076-87-4; 56·1.25HCl, 115076-88-5; 57·HCl, 111188-69-3; 58·HCl, 115076-89-6; 59·2HCl, 111187-90-7; 60, 115076-90-9; 61, 115076-91-0; 62, 115076-92-1; 63·HCl, 111124-66-4; 64·HCl, 111124-65-3; 65·2HCl, 111124-67-5; 66·HCl, 115116-41-1; 67·HCl, 115076-93-2; 68·2.5HCl, 115076-94-3; 69·1.5HCl, 115116-42-2; 70·HCl, 115116-43-3; 71·2HCl, 115076-95-4; 72·HCl, 115092-15-4; 73, 115076-96-5; acrylic acid anhydride with *N,N'*-dicyclohexylcarbamimidic acid, 115076-97-6; *N*-methylcyclohexylamine, 100-60-7; *N*-4-piperidylbenzamide, 33953-37-6; 4-phenyl-4-piperidinol, 40807-61-2; thiomorpholine, 123-90-0; 1*H*-benzotriazole, 95-14-7; ethyl 4-oxo-1,2,3,4-tetrahydro-7-(trifluoromethyl)-3-quinolinecarboxylate, 115076-99-8; theophylline, 58-55-9; 7-[(2-methoxyethoxy)methyl]-3-methylxanthine, 115077-00-4; phthalimide, 85-41-6.

Ab Initio Molecular Electrostatic Potentials of Perillartine Analogues: Implications for Sweet-Taste Receptor Recognition

Thomas J. Venanzi* and Carol A. Venanzi

Chemistry Department, College of New Rochelle, New Rochelle, New York 10801, and Department of Chemical Engineering and Chemistry, New Jersey Institute of Technology, Newark, New Jersey 07102. *Received December 14, 1987*

A model for the recognition of the perillartine analogues has been determined from a consideration of the molecular electrostatic potentials calculated at the ab initio 3-21G level for a select set of biologically active analogues. The model stresses the importance of two regions of negative electrostatic potential. One region, near the oxime moiety, does not vary in shape or value with substitution in the hydrocarbon domain. A second region in the hydrocarbon domain varies in depth, extension, orientation, and shape, depending on the nature of the substituent. The depth, relative position, and orientation of this latter region in the most potent systems (the 1,4-cyclohexadiene analogue and its *p*-methyl derivative) serve as the basis for the optimum recognition pattern of these analogues. The rank order of taste potencies is in general agreement with predictions based on this model. In addition, some conclusions are drawn concerning the receptor–analogue interaction as well as the electrostatic features of the receptor.

I. Introduction

The perillartine analogues are a group of tastants that initiate both sweet and bitter taste response in humans. Many of these compounds display high taste potency with a predominance of either sweet or bitter taste. The perillartine analogues are oximes in the *E* conformation with a CC double bond in conjugation with the CN double bond. A select group of analogues are presented in Chart I (compounds 3–10), along with their taste potency and the ratio of sweet to bitter taste.[1] The mechanism of action of these tastants is not clearly understood, since the receptor for such interactions has never been isolated. However, electronic and topological features as well as hydrophobic contributions[2,3] must play a role in the tastant–receptor interactions.

In previous quantitative structure–activity (QSAR) studies of these analogues, topological information was obtained concerning the important structural parameters (molecular width, length, and thickness) required for taste potency.[4,5] In particular, the appropriate dimensions for

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