the conformations shown in Figure 7 are the only reasonable binding conformations of PCP and acetylcholine. Only the rotation of the piperidine ring differs from that proposed in Figure 1.[2]

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Supplementary Material Available: X-ray structure factors for PAP (7 pages). Ordering information is given on any current masthead page.

The Antihypertensive and Positive Inotropic Diterpene Forskolin: Effects of Structural Modifications on Its Activity

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Four naturally occurring analogues of forskolin were isolated. Forty-nine semisynthetic derivatives were prepared, incorporating structural alterations at the 1-, 6-, 7-, 9-, 11-, and 14/15-positions. Blood pressure lowering properties of 53 compounds were assessed in anesthetized normotensive cats and of 31 compounds in conscious spontaneously hypertensive (SH) rats. The positive inotropic properties of 25 compounds were investigated in an isolated guinea pig atrial preparation. Forskolin was unique among the compounds in its hypotensive activity in cats and in its positive inotropic properties. Although several derivatives displayed oral antihypertensive activity in the SH rats, none was significantly more potent than forskolin. The optimal structural requirements for activity are apparent, since they are found in forskolin itself.

In a program of screening of plants for biological activity, the Indian plant *Coleus forskohlii* Briq. was selected for study of its cardiovascular properties on the basis of its phylogenetic relationship to herbal drugs used in Ayurvedic medicine. Extracts of the roots displayed blood pressure lowering activity in laboratory animals. The major active principle was identified as 7β-acetoxy-8,13-epoxy-1α,6β,9α-trihydroxylabd-14-en-11-one (**1**), which we named forskolin.[1,2]

The biological profile of the compound and its biochemical properties were unravelled through diverse studies at different laboratories in the following chronological sequence. Forskolin displayed blood pressure lowering properties in normotensive and hypertensive laboratory animals, whether administered intravenously or orally. It also had potent, positive inotropic action and a vasodilatory effect. Its mode of action as an antihypertensive agent was related essentially to its peripheral vasodilatory properties.[3] In biochemical studies, forskolin was found to be an activator of adenylate cyclase.[4]

The unusual structural features and biological profile of this natural product provided the impetus to investigate whether it could be transformed into a more potent antihypertensive or cardioactive molecule with a longer duration of activity, especially when administered orally. One approach undertaken in attempts to attain this objective was a study of the manner in which structural modifications of forskolin would affect its biological profile. This study forms the subject of the current paper as described below. Subsequently, forskolin was shown to be unique in its activation of adenylate cyclase, since activation did not require the guanine nucleotide regulatory protein.[5-7] The mode of positive inotropic action of forskolin was postulated to be due to its activation of cardiac adenylate cyclase via an enhanced calcium uptake by the heart muscle cell.[8] The preclinical pharmacological and toxicological data on forskolin were found to be favorable.[9] Forskolin is now scheduled to undergo trials in human volunteers.

In this report we describe the isolation of naturally occurring forskolin analogues, the synthesis of forskolin derivatives, and a comparison of their antihypertensive and positive inotropic properties relative to forskolin.

Chemistry. The compounds listed in Table I are derivatives of forskolin at the 1α-OH, 6β-OH, 7β-OAc, 9α-OH, 11-(>C=O), and $\Delta^{14,15}$ positions. The compounds are so grouped downwards through the table to reflect the sequential progression from single to multiple alterations at the different positions. Compounds **1**, **10**, **33**, and **37**

(1) S. V. Bhat, B. S. Bajwa, H. Dornauer, N. J. de Souza, and H.-W. Fehlhaber, *Tetrahedron Lett.*, 1669 (1977).
(2) V. Shah, S. V. Bhat, B. S. Bajwa, H. Dornauer, and N. J. de Souza, *Planta Med.*, **39**, 183 (1980).
(3) E. Lindner, A. N. Dohadwalla, and B. K. Bhattacharya, *Arzneim.-Forsch.*, **28**, 284 (1976).
(4) H. Metzger and E. Lindner, *IRCS Med. Sci.: Libr. Compend.*, **9**, 99 (1981).
(5) K. B. Seamon, W. Padgett, and J. W. Daly, *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 3363 (1981).
(6) K. B. Seamon and J. W. Daly, *J. Biol. Chem.*, **256**, 9799 (1981).
(7) J. W. Daly, W. Padgett, and K. B. Seamon, *J. Neurochem.*, **38**, 532 (1982).
(8) H. Metzger and E. Lindner, *Arzneim.-Forsch.*, **31**, 1248 (1981).
(9) Preclinical studies were completed at the authors' laboratories and those of Hoechst AG., Frankfurt, in preparation for the filing of an IND application.

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were previously isolated from the roots of *C. forskohlii*.[1] The isolation of 9-deoxyforskolin (**19**) is described here for the first time. The 7-deacetyl derivative (**10**) and the 6-acetate (**37**) isomeric with forskolin were also readily prepared from **1**.[1,10] Alkyl and acyl derivatives of the 1α-, 6β-, 7β-, and 9α-hydroxy groups (compounds **2–5**, **9**, **11–19**, **27–31**, **34–42**, **46–47**, and **51–53**), stereospecifically reduced derivatives of the 11-(>C=O) group (compounds **20**, **43**, **44**, and **49**), and derivatives altered at the 14,15-positions (compounds **21–25** and **50**) were prepared by procedures described by us earlier.[10]

The urethanes **6** and **32** were obtained from **1** and **10**, respectively, by treatment with phenyl isocyanate. The 14-hydroxy-15-amino derivatives (**26** and **45**) were prepared from forskolin 14,15-epoxide (**24**).

The monoamino- and diamino-14,15-dihydroforskolin derivatives **48** and **54** were obtained by catalytic reduction (PtO_2/45 psi) of the oxime derivatives of 1-deoxy-1-oxo-14,15-dihydroforskolin and 1-deoxy-7-deacetoxy-1,7-dioxo-14,15-dihydroforskolin, respectively. Both compounds **48** and **54** were mixture of stereoisomers and were not separated. NMR spectral data for some of the new compounds described in this paper are presented under Experimental Section.

Pharmacology. The cardiovascular profile of forskolin in anesthetized dogs, its hypotensive activity in anesthetized cats, its antihypertensive properties in spontaneously and renal hypertensive rats, its positive inotropic activity, and its probable mode of action have been previously described in some detail.[3,4,8]

The blood pressure lowering properties of the forskolin derivatives were compared with those of forskolin on intravenous administration to anesthetized normotensive cats and on oral administration to conscious spontaneously hypertensive (SH) rats. In the cats, forskolin was administered intravenously through the femoral vein at doses of 25, 50, 100, 250, and 500 μg/kg, each dose being investigated in six animals. The derivatives of forskolin were similarly investigated at doses of 30, 100, 300, 500, and 1000 μg/kg. Mean arterial blood pressure was measured through the femoral artery with a pressure transducer. In the SH rats, applications of a fixed oral dose at 25 mg/kg were made daily to groups of six animals at 24-h intervals for 5 days. Such a method of application is of value for the assessment of compounds of potential use in the chronic treatment of hypertension.[11] On the basis of earlier studies carried out on forskolin in SH rats,[9] wherein doses ranging from 5 to 50 mg/kg were administered, it was observed that a dose of 25 mg/kg displayed a significant fall in systolic blood pressure, which was maximum at 2 h after administration. Readings of blood pressure were taken by the tail-cuff method before the first application and 2 h after each application.

Positive inotropic activity was assessed with a spontaneously contracting, isolated guinea pig left and right auricles preparation.[12] The force of contraction was recorded on a 2-channel Hellige recorder through a strain gauge. The activity of forskolin was investigated at concentrations ranging from 5 to 600 ng/mL. Its derivatives were studied at fixed concentrations of 100 ng/mL. Activity was measured as the percent augmentation of the initial force of contraction.

Results

Blood Pressure Lowering Activity. Anesthetized Cat. Forskolin produces a dose-related fall in anesthetized cats, a dose as low as 25 μg/kg causing a fall in mean arterial blood pressure of 50 mmHg, which returns to normal values after 20 min. Its ED_{20} value 10 min after administration was 38 μg/kg, iv.[13] Of the 4 naturally occurring analogues (**10**, **19**, **33**, and **37**, Table I) of forskolin and the other 49 synthetic derivatives (Table I) investigated, none provided ED_{20} values of less than 500 μg/kg. There were several compounds, however, that produced falls in blood pressure at the higher doses. These results served as one criterion for selection of compounds to be screened in the spontaneous hypertensive rat model described below. Among compounds in this category that attracted our special attention were 7-deacetylforskolin (**10**) and 6-acetyl-7-deacetylforskolin (**37**), which produced falls in blood pressure of about 20%, 10 min after iv administration, at doses of 500 and 300 μg/kg, respectively. Also of interest was the observation that the two compounds, 9-deoxyforskolin (**19**) and 1,9-dideoxyforskolin (**33**), which lack the hydroxy substituents of the respective positions in forskolin, were inactive even at doses of 1 mg/kg.

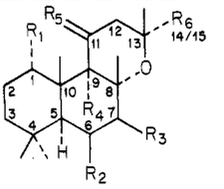
Conscious, Spontaneously Hypertensive (SH) Rat. Thirty-one compounds (Table II) were selected for comparison with forskolin of their antihypertensive properties in SH rats on the basis of their hypotensive potency in the anesthetized cats as described above and on considerations of molecular structure. The difference in systolic blood pressure values recorded prior to the first application of the drug and 2 h after the fifth application is shown in Table II. In the case of forskolin, the difference in systolic blood pressures was -26.0 ± 3.65 mmHg. A fall of about the same magnitude was already observed 2 h after the second application. Readings taken after the third, fourth, and fifth applications were not significantly different from that after the second application. In the case of the forskolin analogues, nearly 50% of them displayed antihypertensive properties. The pattern of the fall in blood pressure produced by the active compounds following five applications was essentially the same as that observed for forskolin.

The limited number of examples of each variation tested do not permit generalized conclusions of SAR trends to be drawn. Furthermore, the comments that can be made about effects of structural variations on potency have, of necessity, to be qualitative, because antihypertensive responses were compared at fixed doses rather than at ED_{50} values.

Among single changes made at one of the variable groups (compounds among **2–24**, Table II), compounds that produced falls in blood pressure comparable to forskolin were those in which the 6-hydroxy group was acetylated (**9**), the 7- acetyl was removed (**10**) or replaced by *n*-alkanoyl groups (**11** and **12**) and the tosyl group (**18**), and the $\Delta^{14,15}$ bond was substituted by oxygen (**21** and **24**). Methylation or silylation of the 1-hydroxy group (**2** and **7**), its conversion to urethane (**6**) or the keto derivative (**8**), replacement of the 7-acetyl group by ethoxycarbonyl (**15**) or diethylaminocarbonyl (**14**), and reduction of the 11-keto group to 11β-hydroxy (**20**) resulted in compounds that produced no significant falls in pressure. The fall produced

(10) S. V. Bhat, B. S. Bajwa, H. Dornauer, and N. J. de. Souza, *J. Chem. Soc., Perkin Trans. 1*, 767 (1982).
(11) N. K. Dadkar, A. N. Dohadwalla, and B. K. Bhattacharya, *J. Pharm. Pharmacol.*, **30**, 58 (1978).
(12) Staff of the Department of Pharmacology, University of Edenburgh, "Pharmacological Experiments on Isolated Preparations", 2nd ed, E & S Livingstone, Edinburgh and London, 1970, pp 112–115.
(13) The ED_{20} value for the standard drug, minoxidil, was determined to be 900 μg/kg, iv.

Table I. Physical Constants of Forskolin and Its Derivatives



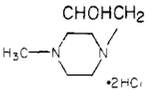
no.	R_1	R_2	R_3	R_4	R_5	R_6	mp, °C (solvent[a])	formula[b]	yield, %
1[c,d]	OH	OH	OCOMe	OH	O	$CH=CH_2$	230–232 (A)	$C_{22}H_{34}O_7$	
2[e]	OMe	OH	OCOMe	OH	O	$CH=CH_2$	192–193 (A)	$C_{23}H_{36}O_7$	
3[e]	OCH_2Ph	OH	OCOMe	OH	O	$CH=CH_2$	132–134 (A)	$C_{29}H_{40}O_7$	
4[e]	OCOMe	OH	OCOMe	OH	O	$CH=CH_2$	203–206 (A)	$C_{24}H_{36}O_8$	
5	OCOPh	OH	OCOMe	OH	O	$CH=CH_2$	178–180 (B)	$C_{29}H_{38}O_8$	72
6	OCONHPh	OH	OCOMe	OH	O	$CH=CH_2$	223–225 (C)	$C_{29}H_{39}NO_8$	48
7	$OSiMe_3$	OH	OCOMe	OH	O	$CH=CH_2$	164–166 (A)	$C_{25}H_{42}O_7Si$	77
8[e]	=O	OH	OCOMe	OH	O	$CH=CH_2$	202–204 (A)	$C_{22}H_{32}O_7$	
9[e]	OH	OCOMe	OCOMe	OH	O	$CH=CH_2$	299–301 (B)	$C_{24}H_{36}O_8$	
10[d]	OH	OH	OH	OH	O	$CH=CH_2$	176–178 (A)	$C_{20}H_{32}O_6$	
11	OH	OH	OCOH	OH	O	$CH=CH_2$	225–227 (A)	$C_{21}H_{32}O_7$	30
12[e]	OH	OH	OCOEt	OH	O	$CH=CH_2$	175–176 (A)	$C_{23}H_{36}O_7$	
13	OH	OH	OCO-*n*-Pr	OH	O	$CH=CH_2$	176–177 (A)	$C_{24}H_{38}O_7$	59
14	OH	OH	$OCONEt_2$	OH	O	$CH=CH_2$	216–219 (A)	$C_{25}H_{41}NO_7$	66
15	OH	OH	OCOOEt	OH	O	$CH=CH_2$	166–167 (A)	$C_{23}H_{36}O_8$	83
16	OH	OH	OCO-3,4,5-$(OMe)_3$-Ph	OH	O	$CH=CH_2$	273–275 (A)	$C_{30}H_{42}O_{10}$	36
17	OH	OH	OSO_2Me	OH	O	$CH=CH_2$	161–162 (A)	$C_{21}H_{34}O_8S$	68
18	OH	OH	OSO_2-4-Me-Ph	OH	O	$CH=CH_2$	187–189 (A)	$C_{27}H_{38}O_8S$	70
19	OH	OH	OCOMe	H	O	$CH=CH_2$	169–170 (B)	$C_{22}H_{34}O_6$	
20	OH	OH	OCOMe	OH	=O, H	$CH=CH_2$	198–199 (D)	$C_{22}H_{36}O_7$	53
21	OH	OH	OCOMe	OH	O	CHO	162–165 (A)	$C_{21}H_{32}O_8$	
22	OH	OH	OCOMe	OH	O	CH_2CH_3	245–248 (A)	$C_{22}H_{36}O_7$	80
23	OH	OH	OCOMe	OH	O	$CHBrCH_2Br$	182–185 (A)	$C_{22}H_{34}Br_2O_7$	50
24[e]	OH	OH	OCOMe	OH	O		155–156 (A)	$C_{22}H_{34}O_8$	
25	OH	OH	OCOMe	OH	O	$CHOHCH_2O$-3-Cl-Ph-CO	204–205 (A)	$C_{29}H_{39}ClO_{10}$	6
26	OH	OH	OCOMe	OH	O	$CHOHCH_2$-*i*-Pr-NH·HCl	208–210[f] (E)	$C_{25}H_{43}NO_8$·HCl	35
27[e]	OCOMe	OCOMe	OCOMe	OH	O	$CH=CH_2$	216–218 (A)	$C_{26}H_{38}O_9$	
28[e]	OCH_2Ph	OH	OH	OH	O	$CH=CH_2$	80–85 (A)	$C_{27}H_{38}O_6$	
29[e]	OCH_2Ph	OH	$OCOCBrMe_2$	OH	O	$CH=CH_2$	158–160 (A)	$C_{31}H_{43}O_7Br$	
30	$OCOCMe_3$	OH	$OCOCMe_3$	OH	O	$CH=CH_2$	169–172 (A)	$C_{30}H_{48}O_8$	55
31	OCOPh	OH	OCOPh	OH	O	$CH=CH_2$	118–120 (A)	$C_{34}H_{40}O_8$	55
32	OCONHPh	OH	OCONHPh	OH	O	$CH=CH_2$	238–242 (A)	$C_{34}H_{42}O_8N_2$	30
33[d]	H	OH	OCOMe	H	O	$CH=CH_2$	162–165 (A)	$C_{22}H_{34}O_5$	
34[e]	O—CO—	OH	OCOMe	O	O	$CH=CH_2$	182–184 (A)	$C_{23}H_{32}O_8$	
35[e]	O—SO—	OH	OCOMe	O	O	$CH=CH_2$	211–216 (F)	$C_{22}H_{32}O_8S$	
36	OCOMe	OH	OCOMe	OH	O	CH_2CH_3	166–169 (G)	$C_{24}H_{38}O_8$	72
37[d]	OH	OCOMe	OH	OH	O	$CH=CH_2$	208–210 (C)	$C_{22}H_{34}O_7$	
38	OH	OCOEt	OH	OH	O	$CH=CH_2$	195–200 (A)	$C_{23}H_{36}O_7$	27
39	OH	OCOMe	OCOEt	OH	O	$CH=CH_2$	229–231 (A)	$C_{25}H_{38}O_8$	44
40	OH	OCOMe	OCO-*n*-Pr	OH	O	$CH=CH_2$	196–198 (F)	$C_{26}H_{40}O_8$	43
41	OH	OCOMe	OCOOEt	OH	O	$CH=CH_2$	177–179 (A)	$C_{25}H_{38}O_9$	68
42[e]	OH	O—CO—O		OH	O	$CH=CH_2$	218–222 (B)	$C_{21}H_{30}O_7$	
43[e]	OH	OH	OH	OH	=O, H, H	$CH=CH_2$	205–209 (B)	$C_{20}H_{34}O_6$	
44[e]	OH	OH	OH	OH	OH	$CH=CH_2$	190–191 (A)	$C_{20}H_{34}O_6$	
45	OH	OH	OH	OH	O		232–234[f] (E)	$C_{25}H_{44}N_2O_7$·2HCl·$2H_2O$	32
46	OMe	OCOMe	OH	OH	O	$CH=CH_2$	195–197 (A)	$C_{23}H_{36}O_7$	48
47	$O(CH_2)_2$-Et_2N·HCl	OCOMe	OH	OH	O	$CH=CH_2$	138–140[g] (I)	$C_{28}H_{47}NO_7$·HCl	45

Table I (*Continued*)

no.	R_1	R_2	R_3	R_4	R_5	R_6	mp, °C (solvent[a])	formula[b]	yield, %
48	$NH_2 \cdot HCl$	OH	OCOMe	OH	—OH, —H	CH_2CH_3	286–288[f] (H)	$C_{22}H_{39}NO_6 \cdot HCl$	11
49[e]	OH	OH	OH	H	—H, —OH	$CH=CH_2$	206–208 (A)	$C_{20}H_{34}O_5$	
50[e]	OH	OCOMe	OH	OH	O	CH_2CH_3	207–211 (A)	$C_{22}H_{36}O_7$	
51[e]	O—	O—CO—O, CO	—O	O		$CH=CH_2$	281–283 (C)	$C_{22}H_{28}O_8$	
52[e]	O—	O—SO—O, SO	—O	O		$CH=CH_2$	205–206 (A)	$C_{20}H_{28}O_8S_2$	
53[e]	β-OH	OCOMe	OH	OH	O	CH_2CH_3	206–207 (A)	$C_{22}H_{36}O_7$	
54	$NH_2 \cdot HCl$	OCOMe	$NH_2 \cdot HCl$	OH	—OH, —H	CH_2CH_3	228–234[f] (H)	$C_{22}H_{40}N_2O_5 \cdot 2HCl \cdot 2H_2O$	19

[a] Solvent of crystallization: A = EtOAc/petroleum ether; B = EtOAc/C_6H_6; C = $CHCl_3$/petroleum ether; D = EtOAc; E = MeOH/OEt_2; F = C_6H_6/petroleum ether; G = petroleum ether; H = MeOH/acetone; I = acetone/petroleum ether. [b] Analytical results were obtained for C and H, as well as for N, Br, Cl, and S when they were present, and are within ±0.4% of the theoretical values. [c] Forskolin. [d] Isolation of these compounds has been reported earlier.[1] [e] Synthesis of these compounds has been reported elsewhere.[10] [f] With decomposition. [g] Of free base.

Table II. Oral Antihypertensive Activity of Forskolin and Its Derivatives

no.	forskolin derivative	Δ BP,[a] mmHg
1	forskolin	−26.0 ± 3.65
2	1-methyl-	0
6	1-(phenylcarbamoyl)-	NS
7	1-(trimethylsilyl)-	0
8	1-deoxy-1-oxo-	0
9	6-acetyl-	−24.3 ± 3.77
10	7-deacetyl-	−24.6 ± 5.69
11	7-deacetyl-7-formyl-	−23.7 ± 5.50
12	7-deacetyl-7-propionyl-	−25.3 ± 3.78
13	7-deacetyl-7-butyryl-	−19.7 ± 6.60
14	7-deacetyl-7-(diethylcarbamoyl)-	0
15	7-deacetyl-7-(ethoxycarbonyl)-	0
18	7-deacetyl-7-(*p*-toluenesulfonyl)-	−27.3 ± 2.76
20	11-deoxo-11-hydroxy-	0
21	13-devinyl-, 13-carboxaldehyde	−22.0 ± 5.24
22	14,15-dihydro-	−15.7 ± 4.50[b]
24	14,15-epoxide	−22.67 ± 3.29
29	1-benzyl-7-deacetyl-7-(2-bromoisobutyryl)-	0
30	7-deacetyl-1,7-dipivaloyl-	0
33	1,9-dideoxy-	0
34	1,9-carbonate	−16.7 ± 3.70
35	1,9-sulfonate	−31.0 ± 1.98
37	6-acetyl-7-deacetyl-	−28.0 ± 4.16
38	6-propionyl-7-deacetyl-	−26.33 ± 3.2[b]
39	6-acetyl-7-deacetyl-7-ethoxycarbonyl-	0
42	7-deacetyl-, 6,7-dicarbonate	−24.67 ± 3.3
43	7-deacetyl-11-deoxo-11β-hydroxy-	−19.3 ± 3.25[c]
45	7-deacetyl-14-hydroxy-15-*N*-methylpiperazino hydrochloride	NS
46	1-methyl-6-acetyl-7-deacetyl-	−25.3 ± 5.38
47	1-[(diethylamino)ethyl]-6-acetyl-7-deacetyl-, hydrochloride	−26.7 ± 13.7
51	7-deacetyl-, 1,9:6,7-dicarbonate	−23.0 ± 3.69
52	7-deacetyl-, 1,9:6,7-disulfonate	0
minoxidil		−51.2 ± 2.3[d]

[a] Δ BP refers to the difference in systolic blood pressure observed prior to the first application and 2 h after the fifth application. [b] Dose administered: 20 mg/kg, po, daily for 5 days. [c] Dose administered: 10 mg/kg, po, daily for 5 days. [d] Dose administered: 5 mg/kg, po, daily for 5 days.

by the 14,15-dihydro derivative (**22**) cannot be compared, as it was applied at a lower dose.

Among derivatives with changes made at more than one site (compounds among **29–52**, Table II), compounds **29** and **30** with benzyl protecting groups and esterase-resistant acyl protecting groups of the 1- and 7-hydroxy functions did not produce falls in blood pressure. 1,9-Dideoxyforskolin (**33**) was also inactive. The 1,9-sulfonate (**35**), 6,7-carbonate (**42**), and 1,9:6,7-dicarbonate (**51**) were as effective as forskolin. The 1,9-carbonate (**34**) and 1,9:6,7-disulfonate (**52**) produced, surprisingly, weaker responses. The 6-acetyl-7-deacetyl derivative (**37**) was also about as effective as forskolin. Increasing the chain length of the 6-alkanoyl group (**38**) or introducing a diethylaminoethyl group at the 1-position in **37** (**47**) resulted in compounds that produced falls in blood pressure comparable to those produced by forskolin. A combination of a 7-ethoxycarbonyl group with the 6-acetoxy group (**41**) had a deleterious effect on activity, similar to **15**.

7-Deacetyl-11-deoxo-11β-hydroxyforskolin (**43**), unlike **20**, applied even at a dose of 10 mg/kg produced surprisingly a fall in blood pressure. In contrast to 1-methylforskolin (**2**) which produced no fall in blood pressure, the isomeric 1-methyl-6-acetyl-7-deacetyl derivative (**46**) was as effective as forskolin. The 14-hydroxy-15-methylpiperazino analogue (**45**) was only weakly active.

Positive Inotropic Activity. The positive inotropic properties of 25 forskolin derivatives (**2**, **8–11**, **13–18**, **33–35**, **37**, **43–49**, and **51–53**) were compared with those of forskolin in the guinea pig atrium model.[12] The concentration at which forskolin augmented the force of contraction by 50% (EC_{50}) was determined to be 2.48×10^{-8} M (1.46×10^{-8} to 4.22×10^{-8} M).[14] Of the derivatives tested, none displayed significant activity at 0.1×10^{-6} g/mL. Three compounds, the 7-butyryl analogue (**13**), the 7-diethylaminocarbonyl analogue (**14**), and 6-acetyl-7-deacetylforskolin (**37**) did, however, show some augmentation of the force of contraction and were about as equipotent as one another. For comparative purposes, EC_{50} values were determined only for 6-acetyl-7-deacetylforskolin (**37**), which was found to be 1.09×10^{-6} M (6.8×10^{-7} to 1.75×10^{-6} M).

Discussion

The results of the studies in the anesthetized cat and the isolated guinea pig atrium models indicate that forskolin is unique among the compounds tested in its hypotensive and positive inotropic properties. In the SH rat model, the compounds tested include those bearing moieties known to alter lipo/hydrophilic properties, to protect metabolically vulnerable groups, or, in general, to

(14) The EC_{50} value for the standard drug, isoprenaline sulfate, was determined to be 1.15×10^{-9} M (6.6×10^{-10} to 1.94×10^{-9} M).

affect factors of absorption, distribution, and elimination pertinent to the modulation of bioavailability on oral administration.[15,16] Among the derivatives are (a) those compounds bearing altered *n*-alkanoyl chain lengths, such as the compound groups (11–13), (9 and 39), (37 and 38); (b) water-soluble compounds 45 and 47; (c) those compounds wherein the C-7 acetyl is replaced, on the one hand, by esterase-facilitating groups, such as the tosyl group (18), or, on the other hand, by esterase-resistant moieties, such as the pivaloyl (30), diethylcarbamoyl (14), ethoxycarbonyl (15), and urethane (6) groups; (d) those compounds in which protection of one or more of the vulnerable C-1, C-6, and C-9 hydroxy groups is attempted through pivaloyl (30), carbonate (34, 42, and 51), and sulfonate (35 and 52) derivatives; and (e) 14,15-dihydroforskolin (22) in which protection of the $\Delta^{14,15}$ bond is attempted by saturation. Although the oral antihypertensive response of forskolin was amenable to alteration through such structural modifications, in that less active molecules or those equipotent with forskolin were produced, no derivative had the ability to produce a response significantly more potent than forskolin.

Forskolin activates cyclic AMP generating systems in a number of mammalian tissues in a rapid and reversible fashion.[5] Derivatives of forskolin have been tested for their ability to stimulate membrane adenylate cyclase from rat brain and heart, as well as cyclic AMP generation in guinea pig brain vesicular preparations. Rank order potencies determined for these derivatives[17] are in general agreement with the qualitative biological results reported in this paper. None of the derivatives had greater potency than that of forskolin. A number of them derivatized at the 6β- or 7β-hydroxy functions were active, in particular the 7-deacetyl-7-propionyl derivative (12) and the 7-deoxy-7-ethoxycarbonyl derivative (15), both of which were about as equipotent as forskolin. 9-Deoxyforskolin (19) retained some activity, but 1,9-dideoxyforskolin (33) was inactive in all the cyclic AMP generating systems tested. Derivatives of the 1α- and 9α-hydroxy functions were also only weak adenylate cyclase activators. Our findings, considered together with these data, suggest one possible obligatory structural requirement for activity of the order shown by forskolin to be displayed, namely, the concomitant presence of both 1α- and 9α-hydroxy groups.

Conclusion

In general, the results of this study and those of the study on the structure–activity correlations of adenylate cyclase activation[17] suggest that the optimal structural requirements for the activity of forskolin are as they are found to be in the natural product. The profile of forskolin, as determined by the comprehensive biological investigations and mode of action studies,[3–9] indicates a clinical potential for the molecule as a novel antihypertensive and positive inotropic agent.[18]

Experimental Section

Chemistry. Melting points were determined with a Kofler hot-stage apparatus. IR spectra were determined with a Perkin-Elmer 157 spectrophotometer for KBr disks. ^{1}H NMR spectra were measured for solution in $CDCl_3$ with a Varian T-60 spectrometer unless mentioned otherwise (Me_4Si as internal standard). For column chromatography, the order of eluents used was hexane, hexane/benzene mixtures, benzene, benzene/ethyl acetate mixtures, and ethyl acetate. Usual workup refers to diluting with water, followed by extracting with $CHCl_3$ or AcOEt, washing the organic layer with water, drying (Na_2SO_4), and evaporating in vacuo. Petroleum ether refers to the fraction of bp 60–80 °C. We used precoated (silica gel 60 F_{254}) TLC plates 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.

9-Deoxyforskolin (19). The compound was isolated from *C. forskohlii*.[1] Following the crystallization of forskolin,[1] the mother liquors were subjected to repeated preparative TLC on silica gel plates with ethyl acetate/benzene (2:8) as solvent. The compound was crystallized from benzene/petroleum ether: mp 169–170 °C; ^{1}H NMR δ 1.0, 1.18, 1.2, 1.41, and 1.56 (3 H each, s, 16-, 17-, 18-, 19-, and 20-Me), 2.15 (3 H, s, COMe), 2.59 (2 H, s, 12-H), 3.50 (1 H, s, 9-H), 4.32 (2 H, m, overlapping signals of 1-H and 6-H), 4.98 (1 H, d, J = 4 Hz, 7-H), 5.1 (m, 2 H, 15-H), 5.94 (1 H, dd, J = 8 and 14 Hz, 14-H); IR ν_{max} 3550, 3430, 1720, 1705 cm^{-1}. Anal. Calcd for $C_{22}H_{34}O_6$: C, 67.30; H, 8.78. Found: C, 66.98; H, 8.69.

1-Benzoylforskolin (5). A solution of forskolin (0.1 g, 0.24 mmol), benzoyl chloride (0.5 mL, 4.3 mmol), and pyridine (0.5 mL) was left overnight at room temperature. The usual workup gave a residue, which was chromatographed over silica gel. The fractions eluted with ethyl acetate/benzene (1:99) were concentrated and crystallized from ethyl acetate/benzene to give 5 (0.09 g, 72%): ^{1}H NMR δ 1.08, 1.30, 1.60, 1.70 (3 + 6 + 3 + 3 H, s, 16-, 17-, 18-, 19-, and 20-Me), 2.20 (3 H, s, COMe), 2.30 and 3.03 (2 H, d, J = 17 Hz, 12-H), 2.38 (1 H, d, J = 3 Hz, 5-H), 4.48 (1 H, dd, J = 4 and 3 Hz, 6-H), 4.78, 5.06, and 5.55 (1 H each, dd, J = 10 and 1.5, 17.5 and 1.5, 10 and 17.5 Hz, respectively, CH=CH_2), 5.5 (1 H, d, J = 4 Hz, 7-H), 5.8 (1 H, br t, $W_{1/2}$ = 10 Hz, 1-H), 8–8.5 (5 H, m, Ar H). Properties of 5 and 30, 31, and 36 prepared in a similar manner are included in Table I.

7-Formyl-7-deacetylforskolin (11). A solution of 7-deacetylforskolin (10; 2.2 g, 5.97 mmol), acetic/formic anhydride (6 mL), and pyridine (10 mL) were stirred at 0–5 °C for 3 h and then at room temperature for 0.5 h. The usual workup gave a residue that was crystallized from ethyl acetate to give colorless needles of 11 (0.7 g, 30%): ^{1}H NMR δ 1.05, 1.30, 1.35, 1.45, and 1.70 (3 H each, s, 16-, 17-, 18-, 19-, and 20-Me), 2.35 and 3.25 (2 H, d, J = 17 Hz, 12-H), 2.1 (1 H, d, J = 3 Hz, 5-H), 4.45 (2 H, m, 1-H and 6-H), 5.75 (1 H, d, J = 4 Hz, 7-H), 4.98, 5.15, and 6.10 (1 H each, dd, J = 10 and 1.5, 17.5 and 1.5, 10 and 17.5 Hz, respectively, CH=CH_2), 8.35 (1 H, s, CHO).

Properties of 11 and of 13–19 and 39–41 prepared in a similar manner are included in Table I.

1-(Anilinocarbonyl)forskolin (6). A solution of forskolin (0.5 g, 1.21 mmol) and phenyl isocyanate (0.35 g, 2.94 mmol) in toluene (25 mL) was refluxed for 20 h. The solvent was distilled in vacuo. The residue after usual workup and crystallization from chloroform/petroleum ether gave colorless crystals of 6 (0.317 g, 48%): ^{1}H NMR δ 1.05, 1.28, 1.32, 1.62, and 1.78 (3 H each, s, 16-, 17-, 18-, 19-, and 20-Me), 2.2 (3 H, s, COMe), 2.38 and 3.2 (2 H, d, J = 17 Hz, 12-H), 4.48 (1 H, m, $W_{1/2}$ = 6 Hz, 6-H), 4.92, 5.16, and 5.94 (1 H each, dd, J = 10 and 1.5, 17.5 and 1.5, 10 and 17.5 Hz, respectively, CH=CH_2), 5.51 (1 H, d, J = 4 Hz, 7-H), 5.6 (1 H, m, $W_{1/2}$ = 10 Hz, 1-H), 7–7.6 (5 H, m, Ar H). Properties of 6 and of 32 prepared in a similar manner are included in Table I.

1-(Trimethylsilyl)forskolin (7). A mixture of forskolin (0.3 g, 0.73 mmol), hexamethyldisilazane (6 mL, 31.7 mmol), trimethylchlorosilane (3 mL, 23.6 mmol), and pyridine (30 mL) was heated at 80–85 °C for 2 h and left overnight. The usual workup gave a residue, which was crystallized from ethyl acetate/petroleum ether to give colorless needles of 7 (0.29, 77%): mp 164–166 °C; ^{1}H NMR δ 0.10 [9 H, s, $Si(CH_3)_3$], 1.03, 1.18, 1.32, 1.48, and 1.68 (3 H each, s, 16-, 17-, 18-, 19-, and 20-Me), 2.10 (3 H, s, COMe), 2.32 and 3.28 (2 H, d, J = 17 Hz, 12-H), 4.45 (2 H, m, 1-H and

(15) E. J. Ariens, in "Drug Design", Vol. II, E. J. Ariens, Ed., Academic Press, New York and London, 1971, Chapter I.
(16) V. Stella, in "Pro-drugs as Novel Drug Delivery Systems" T. Higuchi and V. Stella, Eds. (*ACS Symp. Ser.*, **no. 14**), American Chemical Soceity, Washington, DC, 1975, Chapter I, 1–117.
(17) K. B. Seamon, J. W. Daly, H. Metzger, N. J. de Souza, and J. Reden, *J. Med. Chem.*, **26**, 436 (1983).
(18) N. J. de Souza, A. N. Dohadwalla, and J. Reden, *Med. Res. Rev.*, **3**, 201 (1983).

6-H), 4.85, 5.12, and 5.96 (1 H each, dd, J = 10 and 1.5, 17.5 and 1.5, 10 and 17.5 Hz, respectively, CH=CH_2), 5.52 (1 H, d, J = 4 Hz, 7-H).

13-Devinylforskolin-13-carboxaldehyde (21). Through a cooled solution (−78 °C, dry ice–acetone) of forskolin (0.5 g, 1.21 mmol) in chloroform (50 mL) and pyridine (0.5 mL), a stream of ozonized oxygen was passed for 1 h. Excess ozone was removed by passing a stream of nitrogen for 2 min. Zn dust (1.0 g) and acetic acid (6.0 mL) were added, and the reaction mixture was stirred for 1.25 h at 10 °C (ice–water) and filtered. The filtrate, after the usual workup, gave a residue, which was crystallized from ethyl acetate/petroleum ether to give colorless crystals of the derivative **22** (0.3 g, 60%): mp 162–165 °C; ^{1}H NMR δ 1.40, 1.52, 1.62, 1.82, and 1.86 (3 H each, s, 16-, 17-, 18-, 19-, and 20-Me), 2.48 (3 H, s, COMe), 2.70 and 3.18 (2 H, d, J = 17 Hz, 12-H), 4.7 (2 H, m, 1-H and 6-H), 5.42 (1 H, d, J = 4 Hz, 7-H), 5.45 (1 H, s, 14-H, CHO exists as a hemiacetal with C_9 OH[10]).

Forskolin 14,15-Dibromide (23). To a cooled (0–5 °C) and stirred solution of forskolin (0.1 g, 0.24 mmol) in CS_2 (5 mL) was added a solution of bromine in CS_2 (3%, 1.5 mL, 0.28 mmol), and stirring was continued for 0.75 h at the same temperature and for 2 h at room temperature. The usual workup gave a residue, which was chromatographed over silica gel. The fractions eluted in benzene were crystallized from ethyl acetate/petroleum ether to give colorless crystals of compound **23** (0.07 g, 50%): mp 182–185 °C; ^{1}H NMR δ 1.03, 1.13, 1.23, and 1.44 (3 + 3 + 3 + 6 H, s, 16-, 17-, 18-, 19-, and 20-Me), 2.18 (3 H, s, COMe), 2.38 and 2.72 (2 H, d, J = 17 Hz, 12-H), 3.3–4.2 (3 H, m, 14-H and 15-H), 4.40 (2 H, m, 1-H and 6-H), 5.42 (1 H, d, J = 4 Hz, 7-H).

15-[(*m*-Chlorobenzoyl)oxy]-14-hydroxyforskolin (25). A solution of forskolin (5 g, 12.1 mmol) and *m*-chloroperbenzoic acid (4 g, 23.2 mmol) in chloroform (300 mL) was left overnight at 0–5 °C. The usual workup gave a residue, which was chromatographed over silica gel. Forskolin 14,15-epoxide (**24**)[10] was the major product. The fractions eluted with benzene/ethyl acetate (9:1) were crystallized from ethyl acetate/petroleum ether to give fluffy needles of **25** (0.4 g, 6%): mp 204–205 °C; ^{1}H NMR ($CDCl_3$ + Me_2SO-d_6) δ 1.03, 1.28, 1.40, and 1.66 (3 + 3 + 6 + 3 H, s, 16-, 17-, 18-, 19-, and 20-Me), 2.13 (3 H, s, COMe), 2.32 and 3.50 (2 H, d, J = 17 Hz, 12-H), 3.60 and 4.3–4.48 (5 H, m, 1-H, 6-H, 14-H and 15-H), 5.35 (1 H, d, J = 4 Hz, 7-H), 7.2–8.0 (4 H, m, Ar H).

14-Hydroxy-15-(isopropylamino)forskolin Hydrochloride (26). To a cooled (0–5 °C) and stirred solution of **24**[10] (0.8 g, 1.88 mmol) in methanol (30 mL) was added isopropylamine (7 mL, 82 mmol), and the mixture was stirred at the same temperature for 0.75 h and at room temperature for 1 h. The usual workup gave a residue, which was chromatographed over silica gel. The fractions eluted with ethyl acetate were dissolved in methanol, acidified with 1 N HCl, and evaporated to dryness in vacuo. The residue was reprecipitated from methanol/ether to give **26**: mp 208–210 °C; ^{1}H NMR ($CDCl_3$ + Me_2SO-d_6) δ 1.05, 1.27, 1.43, and 1.65 (3 + 3 + 6 + 3 H, s, 16-, 17-, 18-, 19-, and 20-Me), 1.40 [6 H, d, J = 7 Hz, $CH(CH_3)_2$], 2.13 (3 H, s, COMe), 3.0–4.6 [6 H, m, 1-H, 6-H, 14-H, 15-H and $CH(CH_3)_2$], 5.28 (1 H, d, J = 4 Hz, 7-H). Compound **45** was prepared in an anlogous manner.

1-Deoxy-1-amino-11,14,15-tetrahydroforskolin Hydrochloride (48). A mixture of 1-deoxy-1-oxo-14,15-dihydroforskolin[10] (2.1 g, 5.12 mmol), hydroxylamine hydrochloride (2.1 g, 30.2 mmol), and pyridine (5 mL) was warmed on a water bath for 0.25 h, left overnight at room temperature, diluted with water, and filtered. The solid was washed with water, dried, and crystallized from methanol to give colorless crystals of the 1-oximino derivative (1.88 g), which was dissolved in methanol (150 mL) and hydrogenated (45 psi, 16 h) in the presence of platinum oxide (0.375 g). The reaction mixture was filtered, and the filtrate was acidified with methanolic HCl and evaporated to dryness in vacuo. The residue was macerated with dry acetone. The solid was repeatedly crystallized from methanol/acetone to give a colorless solid, **48** (0.230 g, 11%): mp 286–288 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.0, 1.24, 1.34, and 1.60 (3 + 3 + 6 + 3 H, s, 16-, 17-, 18-, 19-, and 20-Me), 1.94 (3 H, s, COMe), 4.1 and 4.6 (2 + 1 H, m, 1-H, 6-H and 11-H), 5.30 (1 H, m, 7-H). The 1,7-diamino derivative **54** was similarly obtained from the 1,7-dioximino derivative of 6-acetyl-1,7-dideoxy-1,7-dioxo-14,15-dihydroforskolin.[10]

6-Propionyl-7-deacetylforskolin (38). A mixture of **12**[10] (0.75 g, 2.04 mmol), sodium methylate (0.06 g, 1.1 mmol), and dioxane (40 mL) was stirred overnight. The reaction mixture was neutralized (acetic acid) and evaporated to dryness in vacuo and the residue after usual workup was chromatographed over silica gel. The fractions eluted in benzene/ethyl acetate (85:15) were crystallized from ethyl acetate/petroleum ether to give colorless crystals of **38** (0.2 g, 27%): mp 195–200 °C; ^{1}H NMR δ 1.02, 1.08, 1.42, and 1.62 (3 + 3 + 6 + 3 H, s, 16-, 17-, 18-, 19-, and 20-Me), 1.18 and 2.4 (3 + 2 H, t and q, respectively, J = 7 Hz, COEt), 2.35 (1 H, d, J = 3 Hz, 5-H), 2.50 and 3.22 (2 H, d, J = 17 Hz, 12-H), 4.30 (1 H, d, J = 4 Hz, 7-H), 4.68 (1 H, m, 1-H), 5.0, 5.22, and 6.18 (1 H each, dd, J = 10 and 1.5, 17.5 and 1.5, 10 and 17.5 Hz, respectively, CH=CH_2), 5.9 (1 H, dd, J = 4 and 3 Hz, 6-H).

6-Acetyl-7-deacetyl-1-[(diethylamino)ethyl]forskolin Hydrochloride (47). A mixture of **37** (1.0 g, 2.43 mmol),[10] (diethylamino)ethyl chloride (1.5 g, 11.07 mmol), anhydrous acetone (100 mL), and anhydrous K_2CO_3 (4.5 g, 32.6 mmol) was refluxed for 6 h. The acetone was decanted, and the K_2CO_3 cake was washed thoroughly with acetone. The combined acetone solutions were concentrated in vacuo to obtain an oil. The product was chromatographed on silica gel. Elution with benzene/ethyl acetate (90:10) and crystallization from ethyl acetate/petroleum ether gave **47** (free base, 0.6 g, 45%): mp 138–140 °C; ^{1}H NMR δ 0.98, 1.08, 1.40, and 1.55 (3 + 3 + 6 + 3 H, s, 16-, 17-, 18-, 19-, and 20-Me), 2.08 (3 H, s, COMe), 3.40, 2.50, and 0.98 [2 + 6 + 6 H, m, m and t, respectively, J = 7 Hz, CH_2CH_2N $(Et)_2$], 4.21 (1 H, m, 1-H), 4.36 (1 H, d, J = 4 Hz, 7-H), 5.88 (1 H, dd, J = 4 and 3 Hz, 6-H), 4.95, 5.15, and 6.22 (1 H each, dd, J = 10 and 1.5, 17.5 and 1.5, and 10 and 17.5 Hz, respectively, CH=CH_2). The hydrochloride of this compound was prepared by dissolving it in methanol, adjusting the pH of solution to 7 by addition of a solution of methanolic HCl, and evaporating the solution to dryness to give the residue of **47**, which resisted crystallization.

Compound **46** was obtained in an analogous manner.

Pharmacology. Hypotensive Activity in Anesthetized Cats. Cats of either sex weighing between 3.0 and 4.5 kg were anesthetized with a 70 mg/kg iv dose of chloralose in a volume of 7 mL/kg. Mean arterial blood pressure was measured through the femoral artery with a Statham P 23 Db pressure transducer. Mean arterial blood pressure was amplified and registered on a Hellige two-channel recorder. Forskolin and its derivatives were dissolved in propylene glycol (concentration 10 mg/mL), and further dilutions were made in distilled water.

Forskolin was administered intravenously through the femoral vein at various dose levels of 25, 50, 100, 250, and 500 μg/kg. Ten minutes after administration of the compound, fall in mean arterial blood pressure was recorded in six animals. ED_{20} was calculated from log dose–response curves. The doses of the forskolin derivatives administered were 30, 100, 300, 500, and 1000 μg/kg, each dose being studied in three animals. Minoxidil was used as a standard drug. Its ED_{20} was 900 μg/kg iv.

Antihypertensive Assay in Spontaneously Hypertensive (SH) Rats. Male SH rats (250–300 g, 12–16 weeks old), derived from a Wistar–Okamoto strain and obtained from in-house breeding facilities, 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.

Groups of six rats having systolic blood pressures greater than 160 mmHg were selected. Test compounds were administered at a standard dose of 25 mg/kg po given daily for 5 days, as a suspension in 0.5% carboxymethylcellulose in a volume of 10 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 application. Fall in systolic blood pressure was determined as the difference between the posttreatment blood pressure and the initial reading and evaluated statistically with the paired Student's t test. Compounds exhibiting more than 15 mmHg fall in systolic blood pressure and having a level of significance of $p < 0.05$ are included in Table II.

Positive Inotropic Activity. Positive inotropic activity was assessed with a spontaneously contracting, isolated guinea pig left and right auricles preparation.[12] The atria of the freshly sacrificed guinea pig were isolated and suspended in isotonic

Ringer solution at room temperature in an organ bath of 30-mL capacity. The tissue was aerated with a gaseous mixture of 95% O_2 and 5% CO_2. The force of contraction and the rate of contraction were recorded on a two-channel Hellige recorder through a strain gauge. An initial tension of 0.5 to 1 g was given to the preparation. Stabilization time for the preparation was 30 min. Forskolin and its derivatives were dissolved in propylene glycol (concentration 1 mg/mL). Further dilutions were made in distilled water. After taking a basal response, the test compounds were added to the organ bath at doses mentioned below. The volume of the bath was always kept constant. A contact time of 10 min for each compound was given. Inotropic activity was calculated as percent increase over the initial value. EC_{50} values were calculated according to the method of Litchfield and Wilcoxon[19] from four to six dose levels with six atrium preparations per dose. A dose–response relationship for forskolin was achieved with 5, 10, 30, 100, 300, and 600 ng/mL dose levels. The forskolin derivatives were assessed at a dose of 100 ng/mL. Isoprenaline sulfate was used as a standard drug. Its EC_{50} value was 1.15×10^{-9} M (6.6×10^{-10} to 1.94×10^{-9} M).

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Registry No. 1, 66575-29-9; 2, 64657-30-3; 3, 81873-11-2; 4, 64657-22-3; 5, 84048-17-9; 6, 84048-18-0; 7, 84010-19-5; 8, 81873-10-1; 9, 81873-08-7; 10, 64657-20-1; 11, 84048-19-1; 12, 81873-09-8; 13, 84049-21-8; 14, 84010-20-8; 15, 84048-20-4; 16, 84010-21-9; 17, 84010-22-0; 18, 84010-23-1; 19, 84048-28-2; 20, 84048-29-3; 21, 84010-24-2; 22, 64657-24-5; 23, 84010-25-3; 24, 81826-89-3; 25, 81010-26-4; 26, 84010-27-5; 27, 81873-07-6; 28, 81873-12-3; 29, 75879-73-1; 30, 84010-28-6; 31, 84048-21-5; 32, 84048-22-6; 33, 64657-18-7; 34, 81826-81-5; 35, 64657-23-4; 36, 84010-29-7; 37, 64657-21-2; 38, 84048-23-7; 39, 84048-24-8; 40, 84048-25-9; 41, 84048-26-0; 42, 81826-82-6; 43, 81873-13-4; 44, 81873-14-5; 45, 84010-30-0; 45 (free base), 84010-31-1; 46, 84010-32-2; 47, 84010-33-3; 47 (free base), 84048-27-1; 48, 84010-34-4; 49, 81873-15-6; 50, 81873-17-8; 51, 81826-83-7; 52, 84010-35-5; 53, 81873-16-7; 54, 84010-36-6; benzoyl chloride, 98-88-4; phenyl isocyanate, 931-54-4; trimethylchlorosilane, 75-77-4; *m*-chlorobenzoic acid, 535-80-8; isopropylamine, 75-31-0; 1-deoxy-1-oxo-14,15-dihydroforskolin, 64657-25-6; hydroxyamine hydrochloride, 5470-11-1; 1-deoxy-1-oximino-14,15-dihydroforskolin, 84010-37-7; 1,7-dideoxy-1,7-dioximino-6-acetyl-14,15-dihydroforskolin, 84010-38-8; 2-(diethylamino)ethyl chloride, 100-35-6.

(19) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).

Conjugates of Catecholamines. 1. *N*-Alkyl-Functionalized Carboxylic Acid Congeners and Amides Related to Isoproterenol

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A series of functionalized catecholamines (congeners) has been synthesized in which, formalistically, the *N*-isopropyl group of isoproterenol has been extended by a linear alkyl chain of varying length, terminated by a carboxy group or a substituted amide. The compounds were prepared generally via the reductive amination of norepinephrine with a keto acid or a preformed keto amide. An alternate synthesis of the model amide derivatives, involving activation of the carboxylic acid congeners and coupling with amines, was complicated in the case of short-chain derivatives by facile cyclization to lactams. In vitro evaluation of these compounds as potential β-adrenergic agonists has shown that, while the carboxylic acid congeners have relatively low potencies, the model amide derivatives have potencies that are highly dependent on both the length of the alkyl chain and also the nature of the substituent on the amide. In general, aromatic amides are the most potent, although the nature and position of substituents on the aromatic group dramatically influences their potency. The implications of these studies, in terms of general β-adrenergic drug design and also the attachment of the carboxylic acid congeners to carriers, are discussed.

The use of natural or synthetic polymers to modify the activity of biologically active molecules has been the subject of increasing attention in recent years. In particular, the possibility of increasing the therapeutic index of drugs through covalent attachment to polymers offers exciting potential.[1-3] To date, a wide variety of drugs have been attached to polymeric carriers with promising results in many cases, though, as yet, no commercial product has resulted from these studies. This may, in part, be attributed to the difficulty in characterizing completely a conjugate formed between a small drug molecule and a polymeric carrier, which is usually high molecular weight and polydisperse.

Catecholamines and related compounds have been attached to both insoluble carriers, such as porous glass[4] and Sepharose,[5,6] and soluble carriers, such as polypeptides[6,7] and proteins.[6] Although many of these derivatives have shown interesting and reproducible in vitro and in vivo biological activity, none of the systems studied lends itself to a systematic investigation aimed at determining the role of the carrier in affecting the activity of the drug to which it is attached in the conjugate. For example, attempts to characterize previously prepared conjugates of random copolypeptides with isoproterenol have proven to be difficult.[8]

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(1) H. Ringsdorf, *J. Polym. Sci., Polym. Symp.*, **no. 51**, 135–153 (1975).
(2) L. G. Donaruma, *Prog. Polym. Sci.*, **4**, 1–25 (1975).
(3) H.-G. Batz, *Adv. Polym. Sci.*, **23**, 25–53 (1977).
(4) J. C. Venter, J. E. Dixon, P. R. Maroko, and N. O. Kaplan, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 1141–1145 (1972).
(5) M. S. Yong and J. B. Richardson, *Can. J. Physiol. Pharmacol.*, **53**, 616–628 (1975).
(6) K. L. Melmon, Y. Weinstein, B. M. Shearer, H. R. Bourne, and S. Bauminger, *J. Clin. Invest.*, **53**, 22–30 (1974).
(7) M. S. Verlander, J. C. Venter, M. Goodman, N. O. Kaplan, and B. Saks, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 1009–1012 (1976).

0022-2623/83/1826-0492$01.50/0