Forskolin Derivatives. I. Synthesis, and Cardiovascular and Adenylate Cyclase-Stimulating Activities of Water-Soluble Forskolins[1)]

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Water-soluble forskolin and 7-deacetylforskolin derivatives with an aminoacetyl, a 3-aminopropionyl, or a 4-aminobutyryl group at the 6- or 7-position were prepared, and their positive inotropic as well as vasodilative activities were evaluated in anesthetized dogs.

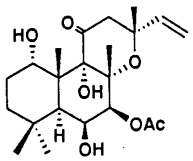
7-Deacetylforskolin (2) and 7-deacetyl-1-silylforskolin (6) were converted to the corresponding 7-chloroacylderivatives (3, 7, 10), which were reacted with amines to obtain 7-aminoacyl-7-deacetylforskolins (4a—f, 9a, b, 11). The 7-acyl substituents migrated to the 6-position with sodium hydroxide in acetonitrile–water to afford 6-aminoacyl-7-deacetylforskolins (12a—f). The 7-position of 12a, d—f was selectively acetylated with acetyl chloride to obtain the corresponding 6-aminoacylforskolins (13a—d).

Among the 6-aminoacylforskolins, 6-(3-dimethylaminopropionyl)forskolin (13b) and 6-(4-dimethylaminobutyryl)forskolin (13d) exhibited potent positive inotropic and vasodilative activities comparable to those of forskolin (1). The activities of 13b and 13d were approximately ten times more potent than those of 7-aminoacyl- and 6-aminoacyl-7-deacetylforskolins (4a—f, 9a, 12a—c, f). 6-Dimethylaminoacetylforskolin (13a) and 6-(3-diethylaminopropionyl)forskolin (13c) were less potent than 1. The effects of the soluble forskolins on adenylate cyclase activity were also examined *in vitro*. 6-Aminoacylforskolins (13a—d) exhibited potent adenylate cyclase-stimulating activity, comparable to that of 1.

Key words forskolin derivative; adenylate cyclase; labdane; inotropism; vasodilation; heart failure

Forskolin (**1**) is a labdane-type diterpene[2)] with potent positive inotropic[3)] and vasodilative[4)] activities. It was isolated from the root of an Indian plant, namely, *Coleus forskohlii*, which has been used among people in India as an therapeutic agent for heart diseases, abdominal colic, respiratory disorders, and other diseases.[5)]

Since the discovery of its unique character as a potent adenylate cyclase stimulant,[6,7)] it has been used worldwide, particularly in biochemical studies. Due to the direct action of forskolin on adenylate cyclase, therapeutic benefit in various diseases, such as congestive heart failure, hypertension, and asthma, has been expected.[8)] Because of its low water solubility (0.0026%), however, the usage of forskolin as a drug has been limited.[9,10)] Accordingly, we synthesized a series of water-soluble *N*-substituted aminoacylforskolins and aminoacyl-7-deacetylforskolins, and evaluated them in an anesthetized dog model similar to that of Khandelwal *et al.*[10)] for positive inotropic and vasodilative activities. We performed a systematic study on the structure–activity relationships regarding the acyl group at the 6- and 7-position, the position of the nitrogen atom on the acyl group, and the bulkiness of the terminal amino group.[11)] We also evaluated the adenylate cyclase-stimulating activity of the synthesized compounds, in guinea-pig heart homogenate.



forskolin (1)

Chart 1

In the present paper, we describe the synthesis of water-soluble forskolin and 7-deacetylforskolin derivatives, and their cardiovascular and *in vitro* adenylate cyclase-stimulating activity.

Synthesis 7-Aminoacetyl-7-deacetylforskolins (**4a—f**) were synthesized by the reaction of 7-deacetylforskolin (**2**)[2a)] with chloroacetyl chloride, followed by the reaction of the resulting 7-chloroacetyl derivative (**3**) with various primary and secondary amines.[10)]

7-(3-Aminopropionyl) compounds (**9a, b**) were synthesized *via* the 1-*tert*-butyldimethylsilyl (TBDMS) derivative. Forskolin (**1**) was reacted with *tert*-butyldimethyl-

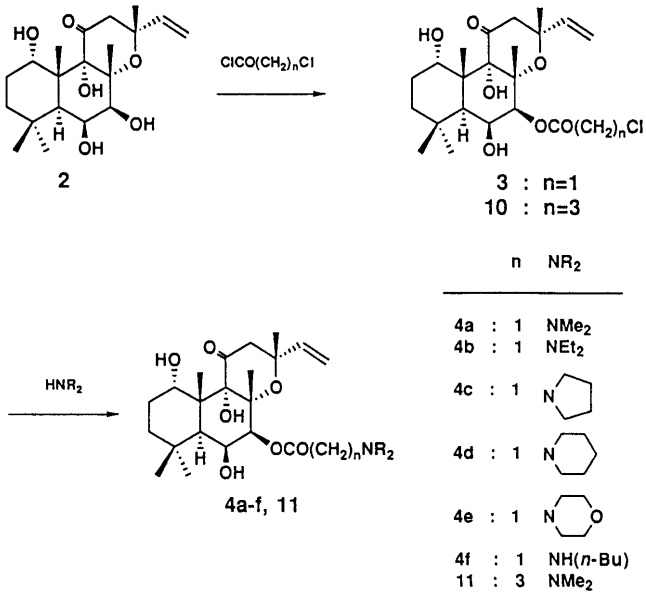


Chart 2

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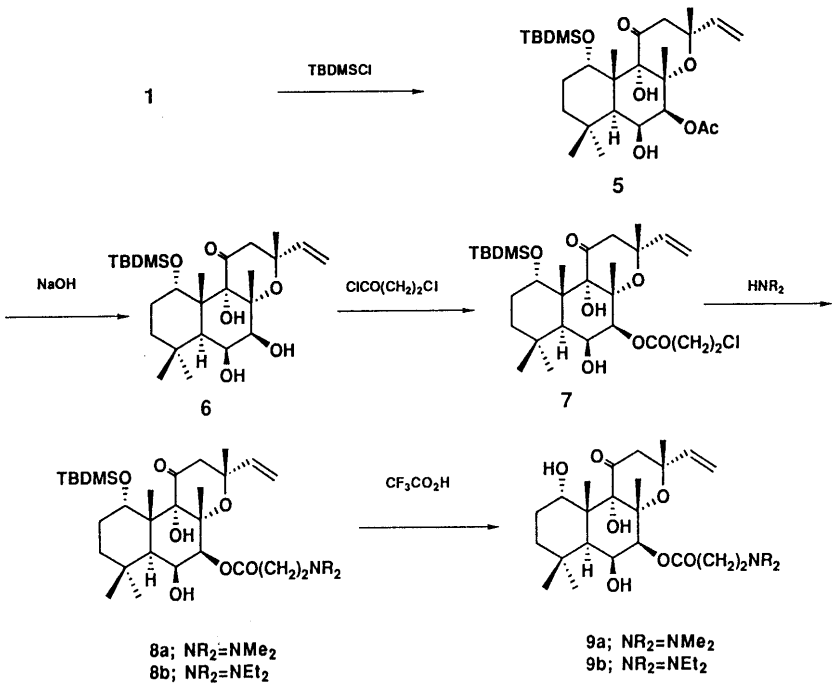


Chart 3

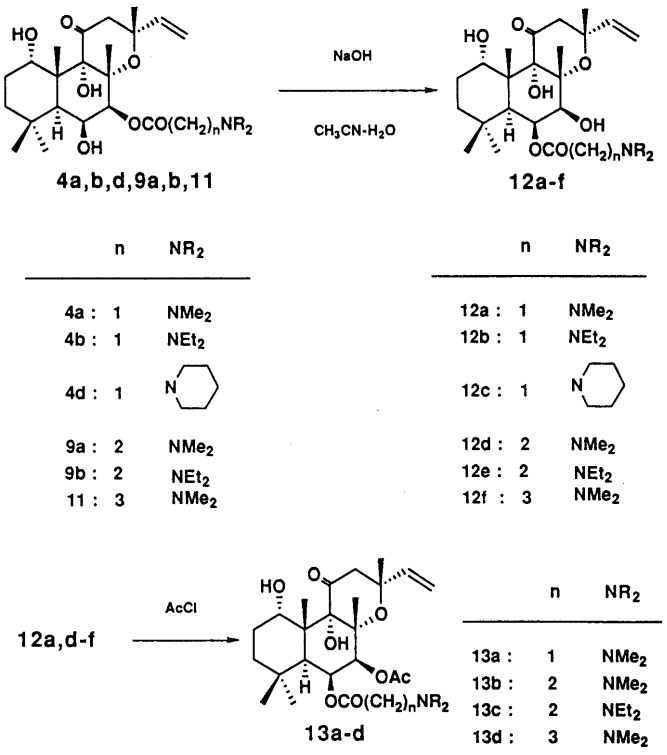


Chart 4

chlorosilane in the presence of imidazole to obtain **5**, which was hydrolyzed with sodium hydroxide in methanol to afford **6**.[10] Treatment of **6** with 3-chloropropionyl chloride gave the 7-(3-chloropropionyl) derivative (**7**), which was converted to the 7-(3-aminopropionyl) derivatives (**8a, b**) by reaction with the appropriate amines. The deprotection of **8a, b** with trifluoroacetic acid in methanol afforded **9a, b**, respectively. The 7-(4-dimethylaminobutyryl) compound (**11**) was synthesized likewise *via* the 7-(4-chlorobutyryl) compound (**10**).[10]

The aminoacyl group at the 7-position of **4a, b, d, 9a, b, 11** migrated to the 6-position upon treatment with sodium hydroxide in acetonitrile–water to afford the 6-aminoacyl compounds (**12a—f**).[11–13] During this process, the formation of a small amount of hydrolysis product of **12** as a by-product was found.

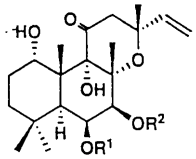
Acetylation of the 6-aminoacyl compounds (**12a, d—f**) with acetyl chloride in the presence of pyridine gave 6-aminoacylforskolins (**13a—d**) due to 7-acetylation.

Solubility in Water For biological testing in the present study, a 0.1% solution of a test compound was sufficient if administered at the dose of 300 μg/kg. Aminoacylated forskolin derivatives (**4a—f, 9a, b, 11, 12a—f, 13a—d**) were found to be soluble at a concentration of more than 0.1% in water at room temperature, when they were mixed with an equimolar amount of hydrochloric acid, and the pH of the aqueous solution was adjusted to between 4 and 5. All the compounds were at least 30 times more soluble in water than forskolin (**1**).

Pharmacological Results and Discussion

Cardiovascular effects of soluble forskolins (**4a—f, 9a, 12a—c, f, 13a—d**) in anesthesized dogs are shown in Table 1.[9,10] Hypotensive activity was measured as an index of vasodilative activity. The positive inotropic and vasodilative activities of these derivatives at the dose of 30 or 300 μg/kg were compared with those of forskolin (**1**) at the dose of 30 μg/kg. Of the derivatives tested at the 300 μg/kg dose, the 7-aminoacetyl derivatives (**4a—f**) were no more effective than **1** at 30 μg/kg. Some of them, at 300 μg/kg, showed approximately the same potency as **1** at 30 μg/kg dose. This indicates that the replacement of the 7-acetyl group of **1** with an aminoacetyl group decreased the potency of positive inotropic and hypotensive activities, independently of the bulkiness or lipophilicity of the terminal amino groups,[11] including **4f** with its

Table 1. Positive Inotropic and Hypotensive Activities, and Adenylate Cyclase-Stimulating Activity of Soluble Forskolins (**4a**—**f**, **9a**, **12a**—**c**, **f**, **13a**—**d**)



Compound	R^1	R^2	Inotropic and hypotensive activities			Adenylate cyclase activity[c] (1 μM)
			Dose (μg/kg, i.v.)	I[a]	H[b]	
4a	H	$COCH_2NMe_2$	300	1.0	1.4	36.6± 3.8 (4)
4b[d]	H	$COCH_2NEt_2$	300	0.4	0.7	18.2± 1.6 (4)
4c[d]	H	$COCH_2N$	300	1.2	1.1	e)
4d[d]	H	$COCH_2N$	300	1.1	0.7	15.8 (2)
4e[d,f]	H	$COCH_2N$ O	300	1.1	1.5	33.5± 4.3 (4)
4f	H	$COCH_2NH(n\text{-Bu})$	300	0.7	0.9	e)
9a	H	$CO(CH_2)_2NMe_2$	300	0.8	1.3	38.0± 2.2 (4)
12a	$COCH_2NMe_2$	H	300	1.0	1.0	21.7± 3.0 (4)
12b[d]	$COCH_2NEt_2$	H	300	1.0	1.1	27.1± 2.5 (4)
12c[d,f,g]	$COCH_2N$	H	300	1.1	0.9	95.8 (2)
12f	$CO(CH_2)_3NMe_2$	H	300	1.0	1.3	27.0± 5.6 (4)
13a	$COCH_2NMe_2$	$COCH_3$	30	0.7	0.3	90.4± 4.7 (6)
13b	$CO(CH_2)_2NMe_2$	$COCH_3$	30	1.2	0.8	107.4± 7.1 (6)
13c	$CO(CH_2)_2NEt_2$	$COCH_3$	30	0.3	0.3	127.9±16.2 (4)
13d	$CO(CH_2)_3NMe_2$	$COCH_3$	30	1.0	0.9	91.1±14.6 (4)
Forskolin (**1**)	H	$COCH_3$	30	1	1	100

a) Positive inotropic activity (mean of 2 experiments). The maximum effect of each soluble forskolin (30 or 300 μg/kg) on dp/dt_{max} was compared with that of forskolin (30 μg/kg) and expressed as a relative value. *b*) Hypotensive activity (mean of 2 experiments). The maximum effect of each soluble forskolin (30 or 300 μg/kg) on mean blood pressure was compared with that of forskolin (30 μg/kg) and expressed as a relative value. *c*) Each value represents the mean±S.E.M. The number of experiments is shown in parenthesis. *d*) Positive inotropic activity in the guinea pig atrium and hypotensive activity in the anesthetized cat were reported in ref. 10. *e*) Not tested. *f*) Cardiovascular effect in the anesthetized dog and acute toxicity in the mouse were reported in ref. 10. *g*) Adenylate cyclase-stimulating properties were reported in ref. 9.

secondary aminoacetyl group. Compound **9a** with a longer acyl chain (3-dimethylaminopropionyl group) at the 7-position was also less potent than **1**. The 6-aminoacetyl derivatives (**12a**—**c**) were equipotent with the 7-aminoacyl derivatives. The 6-(4-dimethylaminobutyryl) derivative (**12f**) with a longer aminoacyl group at the 6-position did not show any more potent activity than **12a**—**c**.

6-Dimethylaminoacetylforskolin (**13a**), the 7-acetyl derivative of **12a**, showed moderate positive inotropic and vasodilative activities even at 30 μg/kg i.v. In contrast to the 7-aminoacyl or 6-aminoacyl-7-deacetylforskolins (**4a**—**f**, **9a**, **12a**—**c**, **f**), 6-aminoacylforskolins with longer aminoacyl groups have more potent activities than **13a**. 6-(3-Dimethylaminopropionyl)forskolin (**13b**) showed more potent activity than **13a**, and was about equipotent with **1** at the same dose of 30 μg/kg i.v. Derivative **13c** with the terminal diethylamino group showed weaker potency than **1**. Derivative **13d** with a 4-dimethylaminobutyryl group at the 6-position also displayed equal potency to **1** at the dose of 30 μg/kg i.v. Compounds **13b** and **13d** are approximately ten times as potent as the 7-aminoacyl- and 6-aminoacyl-7-deacetylforskolins (**4a**—**f**, **9a**, **12a**—**c**, **f**).[10]

Adenylate cyclase-stimulating activity[14] of soluble forskolins was also investigated as shown in Table 1.

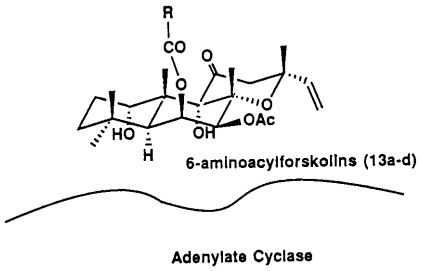


Chart 5

Among the derivatives tested, compounds **12c** and **13a**—**d** showed comparable potency to **1**.

According to our structure–activity studies, addition of a 3-dimethylaminopropionyl or 4-dimethylaminobutyryl group at the 6-position and an acetyl group at the 7-position was found to be optimal with regard to cardiovascular activity.

The α-side of forskolin is thought to bind to adenylate cyclase, while the β-side, the same direction as that of the 6-hydroxy group, is not involved. Thus, the potent adenylate cyclase-stimulating activities of 6-aminoacylated forskolins in our study, particularly of **13a**—**d**, may be related to this putative mode of binding between forskolin and adenylate cyclase.[15]

Conclusion

The present results suggest that 6-(3-dimethylaminopropionyl)forskolin (**13b**) and 6-(4-dimethylaminobutyryl)-forskolin (**13d**) are promising candidates for potent water-soluble positive inotropic and vasodilative drugs with adenylate cyclase-stimulating action. A clinical trial of **13b**·HCl (NKH477) is in progress. Due to the direct action of this compound on adenylate cyclase,[16] it is expected to be effective for the treatment of heart failure, particularly for severe cases accompanied by receptor desensitization.

Experimental

Chemistry Melting points were determined on a Sibata apparatus without correction. Infrared (IR) spectra were measured on a JASCO model A-202 spectrometer, taken as neat film, or Nujol mull as indicated in parentheses. Data are expressed in reciprocal centimeters. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were determined on a JEOL JNM-GX400 (400 MHz) spectrometer. Chemical shifts are reported downfield from tetramethylsilane (TMS) as an internal standard. Data are presented in the form: value of signal (integrated number of protons, peak multiplicity, coupling constant (if any)). Mass spectra (MS) were measured on a Shimadzu DX-300 mass spectrometer operating at 70 eV unless otherwise noted.

Unless otherwise stated, the reaction mixture was evaporated, then the residue was diluted with water, and extracted three times with organic solvent. The extract was washed with saturated brine, dried, and filtered. The solvent was removed by rotary evaporation. The extraction solvent and drying agent are indicated in parenthesis. For column chromatography, Fuji-Davison BW-200 silica gel was used. The chromatographic solvent is presented in parenthesis.

7-Aminoacetyl-7-deacetylforskolins (4a—f) Compounds **4a—f** were prepared from 7-deacetylforskolin (**2**)[2a] *via* 7-chloroacetyl-7-deacetylforskolin (**3**) and the corresponding primary and secondary amines in the same manner as described in the literature.[10]

7-Deacetyl-7-(3-dimethylaminopropionyl)forskolin (9a) 1-(*tert*-Butyldimethylsilyl)-7-deacetylforskolin (**6**) was prepared in the same manner as described in the literature,[10] and was obtained in 99% yield from forskolin (**1**). 3-Chloropropionyl chloride (513 mg, 4.04 mmol) was added dropwise to a mixture of **6** (1.5 g, 4.0 mmol), pyridine (320 mg, 4.05 mmol), and CH_2Cl_2 (10 ml), and the reaction mixture was stirred at room temperature for 5 h. Pyridine (320 mg, 4.05 mmol) and $Cl(CH_2)_2COCl$ (513 mg, 4.04 mmol) were added and the reaction mixture was further stirred at room temperature overnight, then the reaction was quenched with H_2O. Product isolation (AcOEt, $MgSO_4$) gave crude **7** (2.09 g) as an oil.

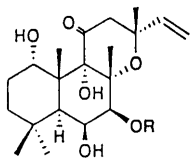
A solution of **7** (1 g) in CH_2Cl_2 (10 ml) was treated with Me_2NH (1 g) under ice cooling, and the reaction mixture was stirred at room temperature for 2 h. The mixture was evaporated to obtain crude **8a** (877 mg) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 5.44 (1H, d, J=4.6 Hz), 4.62 (1H, br s), 4.60 (1H, br s), 2.86 (6H, s), 1.70 (3H, s), 1.45 (3H, s), 1.33 (3H, s), 1.26 (3H, s), 1.05 (3H, s), 0.87 (9H, s), 0.14 (3H, s), 0.02 (3H, s). Trifluoroacetic acid (4 ml) was added to a solution of **8a** (865 mg) in MeOH (10 ml) under ice cooling, and the reaction mixture was stirred at room temperature for 43 h. The mixture was evaporated, and the residue was diluted with dilute HCl, and washed with AcOEt. The aqueous layer was made alkaline with 28% aqueous NH_4OH, and extracted with AcOEt. The organic layer was washed with H_2O, dried over $MgSO_4$, and filtered. The filtrate was evaporated under reduced pressure, and the residue was recrystallized from hexane–CH_2Cl_2 to give **9a** (283 mg, 41% from **6**): mp 150—153 °C. *Anal.* Calcd for $C_{25}H_{41}NO_7$: C, 64.21; H, 8.84; N, 3.00. Found: C, 64.40; H, 8.69; N, 3.17. IR (Nujol) ν_{max}: 3410, 3140, 1705 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.17 (1H, d, J=4.3 Hz), 4.58 (1H, br s), 4.56 (1H, br s), 2.7 (2H, m), 2.63 (1H, m), 2.45 (1H, m), 2.26 (6H, s), 1.77 (3H,s), 1.44 (3H, s), 1.39 (3H, s), 1.27 (3H, s), 1.01 (3H, s). MS *m/z*: 467 (2, M^+), 202 (2), 159 (8), 118 (29), 92 (61), 91 (81), 58 (100). Compound **9b** was obtained in the same manner in 69% yield from **6** as described above using Et_2NH instead of Me_2NH. MS *m/z*: 495 (M^+).

Compound **11** was obtained in the same manner as described in the literature.[10] Yields, melting points, recrystallization solvent, and the chemical formulas of compounds **4a—f**, **11** are listed in Table 2.

7-Deacetyl-6-dimethylaminoacetylforskolin (12a) A solution of **4a** (200 mg, 0.441 mmol) in CH_3CN–H_2O (45 : 55, 20 ml) was treated with 1 N NaOH (0.8 ml, 0.8 mmol), and the mixture was stirred at room temperature for 25 min. Product isolation (AcOEt, $MgSO_4$) gave a residue (242 mg), which was chromatographed on a column of silica gel (25 g, CH_3CN) to obtain **12a** (176 mg, 88%): mp 116—117 °C (hexane–AcOEt). *Anal.* Calcd for $C_{24}H_{39}NO_7$: C, 63.55; H, 8.67; N, 3.09. Found: C, 63.43; H, 8.63; N, 2.95. IR (Nujol) ν_{max}: 3410, 3200, 1750, 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.91 (1H, dd, J=4.4, 2.9 Hz), 4.65 (1H, br s), 4.29 (1H, d, J=4.5 Hz), 3.20, 3.15 (1H each, AB quartet, J=17.1 Hz), 2.36 (6H, s), 1.60 (3H, s), 1.42 (3H, s), 1.39 (3H, s), 1.08 (3H, s), 0.97 (3H, s). MS *m/z*: 453 (5, M^+), 350 (2), 237 (2), 219 (2), 104 (15), 58 (100). Compounds **12b—d, f** were prepared in the same manner as described above from the corresponding 7-aminoacyl-7-deacetylforskolins (**4b, d, 9a, 11**), and their yields, melting points, recrystallization solvents, and chemical formulas are listed in Table 3. 7-Deacetyl-6-(3-diethylaminopropionyl)forskolin (**12e**) was obtained in the same manner from **9b** and was used in the next step without purification.

6-Dimethylaminoacetylforskolin (13a) Pyridine (1 g, 22.1 mmol) and CH_3COCl (751 mg, 9.55 mmol) were added at room temperature in 4 portions to a solution of **12a** (587 mg, 1.29 mmol) in CH_2Cl_2 (20 ml) and

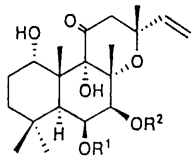
Table 2. 7-Aminoacyl-7-deacetylforskolins (**4a—f, 11**)



Compound	R	Yield[a] (%)	mp (°C)	Recrystn. solvent	Formula[b]
4a	$COCH_2NMe_2$	76	162—166	Hexane–AcOEt	$C_{24}H_{39}NO_7$
4b	$COCH_2NEt_2$	90	130—135[c]	Hexane–AcOEt	$C_{26}H_{43}NO_7$
4c	$COCH_2N$	71	178—182[c]	Hexane–AcOEt	$C_{26}H_{41}NO_7$
4d	$COCH_2N$	47	163—166[c,d]	Hexane–Et_2O	$C_{27}H_{43}NO_7$
4e	$COCH_2N$ O	28	182—185[c,d]	Hexane–AcOEt	$C_{26}H_{41}NO_8$
4f	$COCH_2NH(n\text{-}Bu)$	14	175—178	Hexane–AcOEt	$C_{26}H_{43}NO_7$
11	$CO(CH_2)_3NMe_2$	57	146	Hexane–Me_2CO	$C_{26}H_{43}NO_7$

a) Yield is based on 7-deacetylforskolin. *b*) Analytical data are shown in Table 4. *c*) Melting points and the results of elemental analysis of hydrous or anhydrous monohydrochlorides of **4b—e** are described in ref. 10. **4b**·HCl·H_2O, mp 170—172 °C. **4c**·HCl·1.5H_2O, mp 189—192 °C. **4d**·HCl, mp 194—197 °C. **4e**·HCl, mp 174—178 °C. *d*) mp of **4d**,[10] 162—164 °C (petroleum ether–$CHCl_3$). mp of **4e**,[12] 200—202 °C (hexane–AcOEt).

Table 3. 6-Aminoacyl-7-deacetylforskolins (**12b—d, f**) and 6-Aminoacylforskolins (**13b—d**)



Compound	R^1	R^2	Yield (%)	mp (°C)	Recrystn. solvent	Formula[a]
12b	$COCH_2NEt_2$	H	83	84—86[c]	Hexane–AcOEt	$C_{26}H_{43}NO_7$
12c	$COCH_2N$ ◯	H	32	108—111[b,c]	Hexane–$CHCl_3$	$C_{27}H_{43}NO_7$
12d	$CO(CH_2)_2NMe_2$	H	87	135—136	$(iso\text{-}Pr)_2O$	$C_{25}H_{41}NO_7$
12f	$CO(CH_2)_3NMe_2$	H	38	160—161	$(iso\text{-}Pr)_2O$–AcOEt	$C_{26}H_{43}NO_7$
13b	$CO(CH_2)_2NMe_2$	$COCH_3$	32	184—187	EtOH	$C_{27}H_{43}NO_8$
13c	$CO(CH_2)_2NEt_2$	$COCH_3$	34	101—105	Hexane–Et_2O	$C_{29}H_{47}NO_8$
13d	$CO(CH_2)_3NMe_2$	$COCH_3$	31	240—243	THF	$C_{28}H_{45}NO_8$

a) Analytical data are shown in Table 4. *b*) mp of **12c**,[10] 111—113 °C. *c*) Melting points and the results of elemental analysis of hydrous monohydrochlorides of **12b, c** are described in ref. 10. **12b**·HCl·$2H_2O$, mp 160—163 °C. **12c**·HCl·H_2O, mp 204—205 °C.

Table 4. Analytical Data

Compound	Formula	Calculated			Found		
		C	H	N	C	H	N
4a	$C_{24}H_{39}NO_7$	63.55	8.67	3.09	63.75	8.54	2.89
4b	$C_{26}H_{43}NO_7$	64.84	9.00	2.90	64.52	9.04	3.10
4c	$C_{26}H_{41}NO_7$	65.11	8.62	2.92	64.84	8.43	2.95
4d	$C_{27}H_{43}NO_7$	65.69	8.78	2.84	65.73	8.58	3.03
4e	$C_{26}H_{41}NO_8$	63.01	8.34	2.83	62.77	8.45	2.96
4f	$C_{26}H_{43}NO_7$	64.84	9.00	2.90	64.56	8.72	2.92
11	$C_{26}H_{43}NO_7$	64.84	9.00	2.90	64.71	8.94	3.04
12b	$C_{26}H_{43}NO_7$	64.84	9.00	2.90	64.70	9.07	2.98
12c	$C_{27}H_{43}NO_7$	65.69	8.78	2.84	65.32	8.79	2.74
12d	$C_{25}H_{41}NO_7$	64.21	8.84	3.00	64.01	8.72	2.88
12f	$C_{26}H_{43}NO_7$	64.84	9.00	2.90	64.56	9.12	2.91
13b	$C_{27}H_{43}NO_8$	63.63	8.51	2.75	63.96	8.85	2.76
13c	$C_{29}H_{47}NO_8$	64.78	8.81	2.61	64.57	8.65	2.55
13d	$C_{28}H_{45}NO_8$	64.22	8.66	2.68	64.03	8.91	2.57

the reaction mixture was stirred at room temperature for 7 h. The reaction was quenched with H_2O, then the mixture was made alkaline with saturated aqueous $NaHCO_3$, and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, and filtered. The filtrate was evaporated under reduced pressure, and the residue was chromatographed on a column of silica gel (70 g, hexane–Et_2O, 1 : 3) to give **13a** (444 mg, 69%): mp 190—193 °C (PhMe). *Anal.* Calcd for $C_{26}H_{41}NO_8$: C, 63.01; H, 8.34; N, 2.83. Found: C, 63.05; H, 8.26; N, 2.87. IR (Nujol) ν_{max}: 3100, 1750, 1730, 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.58 (1H, dd, J=4, 2.7 Hz), 5.56 (1H, d, J=4.9 Hz), 4.61 (1H, br s), 3.18 (2H, s), 2.31 (6H, s), 2.04 (3H, s), 1.64 (3H, s), 1.42 (3H, s), 1.35 (3H, s), 1.04 (3H, s), 0.96 (3H, s). MS *m/z*: 495 (17, M^+), 146 (25), 104 (100), 102 (59), 85 (19), 59 (90), 58 (100). Compounds **13b—d** were prepared in the same manner as described above from 6-aminoacyl-7-deacetylforskolins (**12d—f**), and their yields, melting points, recrystallization solvents, and chemical formulas are listed in Table 3.

Pharmacology 1. Positive Inotropic and Hypotensive Activities in Anesthetized Dogs Experiments were performed on beagle or mongrel dogs of either sex, weighing 8.0—14.5 kg. Under sodium pentobarbital (30 mg/kg, i.v.) anesthesia, polyethylene tubes connected to pressure transducers were introduced into the femoral artery and into the left ventricle *via* the carotid artery to measure systemic blood pressure and left ventricular pressure, respectively. Maximum rate of rise in left ventricular pressure (dp/dt_{max}) was obtained with an electric differentiator. All parameters were recorded on polygraphs.

Forskolin was dissolved in 30% dimethylformamide (DMF) saline solution to make a concentration of 1 mg/ml, and water-soluble forskolins were dissolved in equimolar HCl to make a concentration of 1 mg/ml. These solutions were administered intravenously through rubber tubing inserted into the right cephalic vein.

The solvents virtually had no cardiovascular effect if administered alone, without forskolins, at the amount used in the experiments.

The maximum effects of soluble forskolins (30 or 300 μg/kg) on mean blood pressure and dp/dt_{max} were compared with those of forskolin (30 μg/kg) and expressed as relative values. This dose of forskolin increased dp/dt_{max} by 62.5% (n=3) and decreased mean blood pressure by 26.9% (n=3).

2. Adenylate Cyclase Assay A crude enzyme preparation (< 2000 × *g*) obtained from a homogenate of guinea-pig ventricular muscles was used as adenylate cyclase. Adenylate cyclase activity was determined according to the method of Salomon *et al.*[14] in a final incubation volume of 100 μl containing 25 mM Tris·HCl (pH 7.5), 5 mM $MgCl_2$, 20 mM creatine phosphate, 100 U/ml creatine phosphokinase, 1 mM cyclic AMP, 1 mM [^{14}C(U)]ATP (7 cpm/pmol), membrane (100 to 200 μg of protein) and the test compound (1 μM). The assays were carried out at 37 °C for 10 min. Reactions were terminated by adding 100 μl of a stopping solution (pH 7.5) containing 2% sodium dodecylsulfate (SDS), 40 mM ATP and 1.4 mM cyclic AMP and then 50 μl of [^{3}H]cyclic AMP (*ca.* 20000 cpm) was added to each tube to monitor cyclic AMP recovery. Cyclic AMP was separated from ATP by two successive chromatographies on Dowex AG50WX4 and neutral alumina and the radioactivity of the cyclic AMP was determined. The adenylate cyclase-stimulating activity of the test compound (1 μM) was expressed as the mean percentage (±S.E.M.) of that of forskolin (1 μM).

References and Notes

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