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

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

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

Notes

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

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

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

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

Results

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

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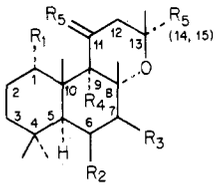
(1) Bhat, S. V.; Bajwa, B. S.; Dornauer, H.; de Souza, N. J. *Tetrahedron Lett.* **1977**, *19*, 1669.
(2) Lindner, E.; Dohadwalla, A. N.; Bhattacharya, B. K. *Arzneim.-Forsch.* **1978**, *28*, 284.
(3) Metzger, H.; Lindner, E. *IRCS Med. Sci.: Libr. Compend.*, **1981**, *9*, 99.
(4) Metzger, H.; Lindner, E. *Arzneim.-Forsch. (Drug Res.)* **1981**, *31*, 1245.
(5) Tandon, J. S.; Dhar, M. M.; Ramakumar, S.; Venkatesan, K. *Indian J. Chem., Sect B*, **1977**, *15B*, 880.
(6) Dubey, M. P.; Srimal, R. C.; Nityanand, S.; Dhawan, B. N. *J. Ethnopharmacol.* **1981**, *3*, 1.
(7) Seamon, K. B.; Padgett, W.; Daly, J. W. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 3363.
(8) Daly, J. W.; Padgett, W.; Seamon, K. B. *J. Neurochem.* **1982**, *38*, 532.
(9) Seamon, K. B.; Daly, J. W. *J. Cyclic Nucleotide Res.* **1982**, *7*, 201.
(10) Seamon, K. B.; Daly, J. W. *J. Biol. Chem.* **1981**, *254*, 9799.
(11) Bhat, S. V.; Bajwa, B. S.; Dornauer, H.; de Souza, N. J. *J. Chem. Soc., Perkin Trans. 1*, **1982**, *3*, 767.
(12) Bhat, S. V.; Bajwa, B. X.; Dahadwalla, A. N.; Dadkar, N. K.; Dornauer, H.; de Souza, N. J. *J. Med. Chem.*, in press.

Table I. Activation of Cyclic AMP Generating Systems by Forskolin and Its Derivatives



no.	R_1	R_2	R_3	R_4	R_5	R_6	EC_{50},[a] μM	% stim[b]	% conv[c]
1	OH	OH	OCOMe	OH	O	$CH=CH_2$	8	100	4.40 ± 0.05
2	OH	OH	OCOEt	OH	O	$CH=CH_2$	15	67 ± 2.2	1.07 ± 0.06
3	OH	OH	OCO_2Et	OH	O	$CH=CH_2$	8	60 ± 1.9	4.01 ± 0.01
4	OH	OH	OCOH	OH	O	$CH=CH_2$	150	52	0.47 ± 0.62
5	OH	OH	OSO_2-4-Me-Ph	OH	O	$CH=CH_2$	>25	5 ± 0.03	0.16 ± 0.02
6	OH	OH	OCO(3,4,5-OMe-Ph)	OH	O	$CH=CH_2$	>25	31 ± 1.2	0.26 ± 0.02
7	OH	OH	$OCONEt_2$	OH	O	$CH=CH_2$	200	59 ± 2.5	0.84 ± 0.03
8	OH	OH	OH	OH	O	$CH=CH_2$	20	51 ± 1.5	1.37 ± 0.01
9	OH	OCOMe	OH	OH	O	$CH=CH_2$	40	31 ± 1.2	1.77 ± 0.05
10	OCOMe	OCOMe	OCOMe	OH	O	$CH=CH_2$	>200	20 ± 1.0	0.47 ± 0.62
11	H	OH	OCOMe	H	O	$CH=CH_2$		1 ± 0.2	0.18 ± 0.01
12	OH	OH	OCOMe	H	O	$CH=CH_2$	100	46 ± 4.2	0.84 ± 0.05
13	O—SO	OH	OCOMe	O	O	$CH=CH_2$	>200	28 ± 3.1	1.2 ± 0.1
14	O—CO—CO	O	O	O	O	$CH=CH_2$		1 ± 0.2	0.25 ± 0.04
15	OH	OH	OCOMe	O	O	$CHCH_2OH$		0 ± 0.2	0.18 ± 0.01
16	O	OH	OCOMe	OH	O	$CH=CH_2$	200	47 ± 2.0	0.76 ± 0.01
17	O	O	OCOMe	OH	O	$CH=CH_2$		0 ± 0.2	0.17 ± 0.01
18	OH	OH	OCOMe	OH	OH, H	$CH=CH_2$	50	49 ± 1.4	0.97 ± 0.02
19	OH	OH	OH	H	H, OH	$CH=CH_2$		1.6 ± 0.01	0.41 ± 0.04
20	OH	OH	OCOMe	OH	O	CH_2CH_3	100	49 ± 1.4	0.97 ± 0.02
21	OH	OH	OCOMe	OH	O	CH—CH_2 (O)		5 ± 0.2	0.22 ± 0.01

[a] The EC_{50}'s for activation of rat cerebral cortical adenylate cyclase were determined as described under Experimental Section. Some compounds (4–7, 10, 12, 13, 16, and 20) did not achieve full activation at their maximal doses. The EC_{50} values for these compounds were estimated as the concentration required to achieve 50% of the maximal activity elicited by 200 μM forskolin (see Discussion). [b] The percent stimulation of heart adenylate cyclase was determined in rabbit heart membranes for 5 μM of each derivative as compared to 5 μM of forskolin. This concentration of forskolin stimulated the enzyme from a basal level of 18 to 110 pmol min^{-1} mg^{-1} as described in ref 4. Each value is the mean plus or minus from at least three determinations. [c] The percent conversion of total labeled nucleotides to cyclic AMP was determined in guinea pig cerebral cortical vesicular entities as described in ref 13 for 200 μM of each compound. The basal level of conversion is 0.18%, and all values are the mean plus or minus SEM from at least triplicate determinations.

from 95 to 620 pmol min^{-1} mg^{-1}. The concentration required for half-maximal activation is 8 μM. Two derivatives of forskolin display potencies similar to that of native forskolin. These are the 7-propionyl (**2**) and the 7-(ethyl carbonate) (**3**) derivatives. Maximal stimulation is achieved by each derivative, with the concentration for half-maximal stimulation being 15 μM for the 7-propionyl derivative and 8 μM for the 7-(ethyl carbonate) derivative. Other substitutions at the 6- or 7-hydroxy functions resulted in derivatives that were less potent than native forskolin (Table I). The 7-formyl derivative (**4**) was more potent than the 7-tosyl (**5**) or 7-trimethoxybenzoyl (**6**) derivatives. The 7-tosyl (**5**) and 7-trimethoxybenzoyl (**6**) derivatives were not soluble above about 50 μM. At this concentration, they stimulate the enzyme about 2-fold. The 7-deacetyl derivative (**8**) was somewhat more potent ($EC_{50} \approx 20$ μM) than the 6-acetyl-7-deacetyl derivative (**9**) ($EC_{50} \approx 40$ μM). Reduction of the carbonyl group, resulting in an 11β-hydroxy derivative (**18**), also reduced activity ($EC_{50} \approx 50$ μM) when compared to forskolin. The 11α-hydroxy-7-deacetyl-9-deoxy derivative (**19**) was totally inactive. Reduction of the double bond yielded dihydroforskolin (**20**), which was less potent ($EC_{50} \approx 100$ μM) than forskolin. The 14,15-epoxy derivative of forskolin (**21**) was inactive. The $\Delta^{14,15}$ bond, thus, appears relatively critical for biological activity. The 1- and 9-hydroxy groups of forskolin are important for biological activity. Thus, the 1,9-dideoxy derivative (**11**) of forskolin does not stimulate adenylate cyclase. The 1,9-sulfite (**13**) has greatly reduced activity, and the 6,7:1,9-dicarbonate (**14**) is totally inactive. Formation of a 9-ether linkage (**15**) or formation of a 1,6-diketo derivative (**17**) also abolishes activity. Acetylation at both the 1- and 6-hydroxy groups (**10**) reduces activity, but this 1,6-diacetyl derivative still stimulates adenylate cyclase ($EC_{50} > 200$ μM). The 1-keto derivative (**16**) is also partially active. Thus, derivatization of the 1-hydroxy group does not appear to affect the activity as much as derivatization at the 9-hydroxy group. However, the 9-hydroxy group is not absolutely essential for activity, since the 9-deoxy derivative (**12**) still retains some activity ($EC_{50} \approx 100$ μM).

The forskolin derivatives also were tested for their ability to activate rabbit heart adenylate cyclase. All compounds were tested at a single concentration of 5 μM and were compared to the level of stimulation elicited by 5 μM forskolin (Table I). Similar effects were obtained for the heart adenylate cyclase as had been found in the more detailed studies with the brain enzyme. The 7-(ethyl carbonate) (**3**) and 7-propionyl (**2**) derivatives were the most active derivatives as had been observed for the brain

enzyme. Other derivatives at the 7-hydroxy function exhibited reduced activity when compared to forskolin. Derivatives at the 1- and 9-hydroxy functions also were less active, in agreement with the results for the brain enzyme. Although dose–response curves were not run for the cardiac enzyme, it appears that the same rank order of potency pertains for both cardiac and brain adenylate cyclase.

The inactive derivatives of forskolin were tested for their ability to inhibit the stimulation of rat cerebral cortical adenylate cyclase by 5 μM forskolin (data not shown). These inactive derivatives were tested at 50 and 200 μM. None of the inactive compounds acted as antagonists (data not shown) of the forskolin-stimulated adenylate cyclase. The same is true for the adenylate cyclase from rat heart membranes. Activation of this system with 1 μM forskolin was not influenced by 1,9-dideoxyforskolin in concentrations up to 100 μM. Derivatives that alone were able to activate the enzyme had additive effects with forskolin when tested together with 5 μM forskolin. However, enzyme maximally stimulated by forskolin was not further stimulated by any of the other active derivatives (data not shown).

Forskolin derivatives also were tested for their ability to activate cyclic AMP generating systems in guinea pig cerebral cortical vesicular preparations[13] (Table I). Forskolin stimulates cyclic AMP generation approximately 30-fold at a concentration of 200 μM. The basal levels of conversion of the labeled adenine nucleotides, within the vesicular preparation, to cyclic AMP were increased from 0.16 to 4.4% by 200 μM forskolin. This occurs with an EC_{50} of about 20 μM. The 7-(ethyl carbonate) (**3**) derivative of forskolin produces almost the same level of stimulation as forskolin. However, the 7-propionyl (**2**) derivative, which is almost equipotent with forskolin in activation of membrane adenylate cyclase, causes less of a stimulation in the vesicular preparation than either forskolin or the 7-(ethyl carbonate) derivative. Other derivatives at the 6- or 7-hydroxy positions exhibit a similar rank order for activation of cyclic AMP systems in vesicular preparations as observed for the membrane adenylate cyclases. Derivatives at the 1- and 9-hydroxy functions are less active, in agreement with the results with the membrane adenylate cyclase systems.

Discussion

Forskolin is a unique tool for studying the role of cyclic AMP in eliciting a physiological response. This derives from the ability of forskolin to activate cyclic AMP generating systems in tissues from most, if not all, higher organisms.[3,4,9] Activation of cyclic AMP generating systems in intact cells by forskolin occurs via the interaction of the diterpene with the enzyme adenylate cyclase. This activation does not require the guanine nucleotide regulatory subunit of the enzyme nor does it require a membrane structure or detergent micelle.[10] Activation of adenylate cyclase by forskolin has been proposed to derive from an interaction of forskolin with the catalytic subunit of the enzyme or an associated protein.[10] It was the aim of the present study to define the structure–activity relationships of forskolin in activating adenylate cyclase.

A number of derivatives involving the 1-, 6- and 7-hydroxy groups still retain significant activity. Thus, a number of 7-acyl derivatives are active, with the 7-(ethyl carbonate) and 7-propionyl being almost as potent as forskolin. The 1,6-diacetyl derivative of forskolin is also active. A 7-acyl group is not critical for activity, as the 7-deacetyl derivative is also active. The biological activity of forskolin is extremely sensitive to the disposition of the 1- and 9-hydroxy function. Derivatization at these positions leads to either inactive compounds (compounds **11**, **14**, **15**, **17**, and **19**) or a marked loss in potency (compound **13**). It would appear that the region below the ring system (α) is an important determinant for forskolin's ability to activate adenylate cyclase. Reduction of the 11-keto group to the 11β-hydroxy compound with the hydroxy function above the plane of the ring does not abolish activity, and thus the keto function is not critical for activity. The reduction of the 14,15 double bond also reduces activity, while epoxidation at this position results in an inactive derivative. This vinyl substituent lies on the α face of the ring system.

A number of derivatives of forskolin have been tested for their antihypertensive and positive inotropic actions.[12] The biological activities of the derivatives are qualitatively similar to the biochemical results reported in this paper. Derivatives at the 6- or 7-hydroxy function are less active than forskolin, while derivatization at the 1- or 9-hydroxy function results in a marked decrease in activity. These results are fully consistent with the proposal that the pharmacological effects of forskolin derive from its ability to activate cyclic AMP generating systems.

The guinea pig vesicular preparation has been shown to take up radioactive adenine. Hormones, then, can convert a percentage of labeled intravesicular adenine nucleotides to cyclic AMP.[13] Such an assay therefore measures the production of cyclic AMP within intact vesicles. Furthermore, this preparation has been shown to maintain a normal membrane potential and to depolarize in response to veratridine, batrachotoxin, and high potassium concentrations and is, thus, a simple model for an intact cell.[14] The response of the cyclic AMP generating system in this vesicular preparation was therefore used to ascertain the relative activity of the various forskolin derivatives in intact systems. The rank order of potency for activation of adenylate cyclase in crude membranes is very similar to that for the activation of adenylate cyclase in the vesicular preparation. This would suggest that the ability of the derivatives tested in this study to activate cyclic AMP generation in intact preparations is not wholly determined by their relative access to the intravesicular (intracellular) side of the plasma membrane.

It was hoped that some of the inactive derivatives of forskolin would act as antagonists or partial agonists and, thus, provide useful research tools. However, none of the derivatives inhibited activation by forskolin of adenylate cyclase in either the crude membrane system or the vesicular preparation. Many of the partially active compounds did not achieve full activation at 200 μM. Since forskolin and many of its derivatives are not soluble above 200 μM in aqueous media, it is difficult to determine if any of the partially active compounds are true partial agonists. The activation of adenylate cyclase by the partially active derivatives was, however, additive with that of forskolin only up to the maximal level of stimulation attained with forskolin. Thus, activation by these derivatives probably occurs at the same site(s) as forskolin.

The structure–activity data indicate that relatively minor modifications of the forskolin structure can completely abolish activity. For example, the 1,9-dideoxy and the 14,15-epoxy derivatives of forskolin are totally inactive. These modifications would not be expected to drastically affect the bulk lipophilicity of the compound and would suggest that forskolin's site of action is determined by

(13) McNeal, E. T.; Creveling, C. R.; Daly, J. W. *J. Neurochem.* **1980**, *35*, 338.

rather specific interactions rather than a nonspecific interaction at hydrophobic regions. This is consistent with the inability of the 1,9-dideoxy derivative to antagonize forskolin's activation of adenylate cyclase.

One of the aims of this study was to determine if the potency of forskolin in activating adenylate cyclase could be increased by appropriate derivatization. Although a number of derivatives of forskolin were active, there were none with greater potency than the native molecule. The results of this study do, however, allow characterization of the binding site in terms of the rank order of potency of the partially active species. This could be used to establish a correlation between the initiation of a physiological process induced by forskolin and its derivatives with the activation of adenylate cyclase. Furthermore, a number of inactive derivatives of forskolin are identified that are not grossly different in structure from forskolin, and these can be used as controls in assessing nonspecific effects due to the diterpenoid structure. The similarity of the data on the activity of forskolin derivatives with brain and heart adenylate cyclase, while limited, suggests that the forskolin site may be relatively invariant in different tissues and species (rat, rabbit and guinea pig).

Experimental Section

Biological Test Procedures. Rat cerebral cortex membranes were prepared as described previously.[7] Briefly, rat cerebral cortex was removed from male Sprague–Dawley rats (150–175 g) and chilled briefly in ice-cold Krebs–Ringer bicarbonate glucose buffer (KRB). The tissue was homogenized in ice-cold (50 mM) Tris-HCl buffer, pH 7.5, 0.1 mM $CaCl_2$, in a Dounce homogenizer. The homogenate was centrifuged at 10000*g* for 10 min, and the pellet was washed once in ice-cold buffer, centrifuged, and resuspended in ice-cold 50 mM Tris-HCl, pH 7.5, 0.1 mM $CaCl_2$. Fresh membranes were used in all experiments. Adenylate cyclase experiments were carried out as described previously.[10] Incubations were in a total volume of 250 μL containing 50 mM Tris-HCl buffer, pH 7.5, 1.0 mM 3-isobutyl-1-methylxanthine, 5 mM $MgCl_2$, 0.1 mM ATP, and 0.2 mM EGTA. Each assay contained 2 μCi of [α^{32}-P]ATP and a nucleotide-regenerating system of 5 units creatine phosphokinase and 2 mM creatine phosphate. Assays were initiated by the addition of 25 μL of membranes (~100 μg of membrane protein), were carried out at 30 °C for 10 min, and were terminated by the addition of 0.5 mL of 10% trichloroacetic acid. Carrier cyclic AMP, 0.25 mL of 1 mM cyclic AMP, was added, and radioactive cyclic AMP was isolated and analyzed as described by Salomon et al.[15] Assays were carried out in triplicate. The EC_{50}'s for activation were taken from at least two determinations over a concentration range from 0.01 to 200 μM of the derivative. The EC_{50}'s for the determinations did not vary more than 20%. Adenylate cyclase activity in rabbit cardiac membranes was assayed as described.[4]

A brain vesicular preparation was obtained from male NIH strain guinea pigs (300 g) as described by McNeal et al.[13] Briefly, the method was as follows. Immediately upon removal, the brain was placed on an ice-cooled petri dish and slices of gray matter were cut by hand from the cerebral cortex. The slices weighing about 1.0 g were homogenized by hand in an all-glass homogenizer containing 10 volumes (10 mL/1 g wet weight) of Krebs–Ringer–bicarbonate–glucose buffer (KRB), pH 7.4, preequilibrated with a mixture of 95% O_2–5% CO_2. This homogenate was then centrifuged at 1000*g* for 15 min at 4 °C, and the supernatant was discarded. An aliquot of the vesicular preparation (5 mL) was incubated with 125 μCi of [^{3}H]adenine (specific activity 16.6 Ci/mmol) for 30 min at 37 °C in KRB. The preparation was centrifuged for 5 min at 1000*g*, washed once with cold buffer, and resuspended in 5 mL of cold buffer. This preparation was postincubated for 15 min at 37 °C, washed once, and resuspended in 5 mL of cold KRB. Aliquots (0.2 mL) of the preparation were placed in test tubes and allowed to equilibrate at 37 °C for 10 min. Agents were added in 50-μL aliquots, and the tubes were incubated an additional 10 min at 30 °C. The incubation was stopped with 0.5 mL of 10% trichloroacetic acid, and 0.25 mL of 2 mM cyclic AMP was added as carrier. After centrifugation, 50 μL of the supernatant was removed to determine the amount of total radioactive adenine nucleotides. Radioactive cyclic AMP was determined in the remaining 0.95 mL of supernatant by the method of Salomon et al.[15] Activity is expressed as the percent conversion of total radioactive adenine nucleotide to radioactive cyclic AMP and is the average plus or minus SEM of duplicate determinations.

Registry No. 1, 66575-29-9; 2, 81873-09-8; 3, 84048-20-4; 4, 84048-19-1; 5, 84010-23-1; 6, 84010-21-9; 7, 84010-20-8; 8, 64657-20-1; 9, 64657-21-2; 10, 81873-07-6; 11, 64657-18-7; 12, 84048-28-2; 13, 64657-23-4; 14, 81826-83-7; 15, 84011-60-9; 16, 81873-10-1; 17, 84011-61-0; 18, 84048-29-3; 19, 81873-15-6; 20, 64657-24-5; 21, 81826-89-3; adenylate cyclase, 9012-42-4; cyclic AMP, 60-92-4.

(14) Creveling, C. R.; McNeal, E. T.; McCulloh, D. H.; Daly, J. W. *J. Neurochem.* **1980**, *35*, 922.
(15) Salomon, Y.; Londos, C.; Rodbell, M. *Anal. Biochem.* **1974**, *58*, 541.

Ferric Ion Sequestering Agents. 11. Synthesis and Kinetics of Iron Removal from Transferrin of Catechoyl Derivatives of Desferrioxamine B[1]

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Two catechoyl derivatives of desferrioxamine B have been synthesized. The more soluble *N*-(2,3-dihydroxy-4-carboxybenzoyl)desferrioxamine B derivative was found to remove iron from the human iron transport protein transferrin with a pseudo-first-order rate constant of 8.2×10^{-4} min^{-1} (0.2 mM ligand concentration). These results indicate that, unlike desferrioxamine B (Desferal) itself, the synthetic monocatechoyl derivative *is* kinetically able to remove transferrin-bound iron. The possible use of these derivatives in the treatment of transfusion-induced iron overload is discussed.

In previous papers in this series we have described the synthesis and characterization of catechol-based iron chelating agents.[2,3] The purpose of this research has been the formulation of drugs capable of removing excess iron from the bodies of iron-overloaded patients and which (ideally) are orally effective. There is an acute need for such agents in the treatment of the iron overload that

(1) Previous paper in this series: Kappel, M. J.; Raymond, K. N. *Inorg. Chem.* **1982**, *21*, 3437–3442.
(2) Weitl, F. L.; Harris, W. R.; Raymond, K. N. *J. Med. Chem.* **1979**, *22*, 1281–1283.
(3) Weitl, F. L.; Raymond, K. N.; Durbin, P. W. *J. Med. Chem.* **1981**, *24*, 203–206.

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