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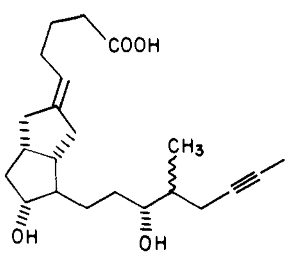
Communications to the Editor

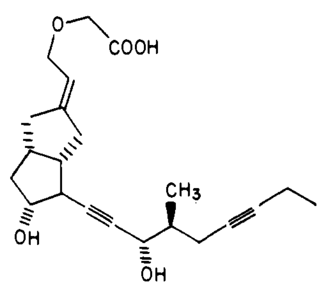
Synthesis of a New Chemically and Metabolically Stable Prostacyclin Analogue with High and Long-Lasting Oral Activity[1]

Sir:

Due to the inherent instability of natural prostacyclin (PGI_2)[2] toward hydrolytic conditions, we have been engaged in a program to develop chemically stable prostacyclin analogues.[3] Our first carbacyclin analogue iloprost[4] (1) showed a nearly identical profile of action and comparable potency to natural PGI_2[5] in pharmacological and clinical studies. In contrast to natural PGI_2, iloprost (1)



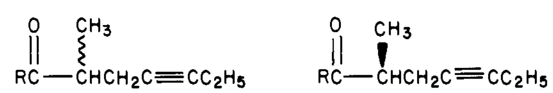
1 (iloprost)



2 (ZK 96 480)

is orally active in man with a biological half-life of 20–30 min, showing inhibition of ex vivo ADP-induced platelet aggregation at threshold doses of 0.5 μg/kg and vasodilating effects at doses of ca. 2–3 μg/kg lasting for ca. 30 min.[15b] This relatively short duration of action after oral application is due to a rapid metabolism of iloprost (1), primarily by β-oxidation of the upper side chain.[6] A longer duration of action of an orally active prostacyclin analogue would facilitate its clinical application. We have therefore modified iloprost (1) to impede the metabolic inactivation while preserving its high intrinsic activity. As a first modification, we replaced the methylene group in the 3-position of 1 by an oxygen atom to prevent the β-oxidation of the upper side chain. The resulting decrease in intrinsic activity was compensated for by modification of the lower side chain. We converted the 13,14-double bond into a triple bond, introduced a further methyl group at C-20, and synthesized selectively the pure 16(*S*)-methyl diastereomer. These modifications resulted in the structure of 2 (ZK 96 480), a carbacyclin analogue with a biological activity at least as high as that of prostacyclin and iloprost.

The synthesis of 2 started with the preparation of the lower side chain by resolving racemic 2-methyl-4-heptynoic acid (**3**).[7] By application of the method of Helmchen et al.,[8] **3** was converted with phosphorus trichloride into the acid chloride **4**, which gave with D-(−)-α-phenylglycinol a pair of diastereomeric amides. After chromatographic separation on SiO_2, the more polar amide **5** (mp 124 °C) was hydrolyzed with 3 N H_2SO_4 in dioxane to furnish the optically pure 2*S*-configurated acid **6** ($[\alpha]_D$ −1.2° (*c* 1, EtOH), bp 128 °C (12 mm)). The 2*S* configuration of **6** was determined by hydrogenation of **6** to 2(*S*)-methylheptanoic acid ($[\alpha]_D$ +17.7° (*c* 1, EtOH)), which was compared with 2-methyl-alkanoic acids of known absolute configuration.[9] Esterification of **6** with diazomethane followed by reaction of the methyl ester **7** ($[\alpha]_D$ +12.2° (*c* 1, EtOH), bp 70 °C (12 mm)) with the lithium salt of ethyl methylphosphonate afforded the optically pure phosphonate **8** ($[\alpha]_D$ +35.3° (*c* 1, EtOH), bp 123 °C (0.3 mm)).



3, R = OH
4, R = Cl

5, R = NHC*HPh (HO)
6, R = OH
7, R = OCH_3
8, R = $(C_2H_5O)_2P(O)CH_2$

Condensation of the phosphonate **8** with the readily available optically pure bicyclic aldehyde **9**[3,4] (NaH, DME,

(1) Prostaglandin Analogues. Part 9. For part 8, see: Bennua, B.; Dahl, H.; Vorbrüggen, H. *Synthesis*, in press.
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Table I. Effects on in Vitro Platelet Aggregation IC_{50} Values for Inhibition of Platelet Aggregation in PRP-Induced by Different Stimuli

	IC_{50}, nM			
substance	ADP (0.5 × 10^{-6} M), human PRP	ADP[b] (1.25 × 10^{-6} M), rat PRP (Wistar)	thrombin[d] (0.1 IU/mL), human PRP	collagen[e] (0.66 μg/mL) human PRP
2	0.64 ± 0.04[a]	3.20 ± 0.22[c]	0.13	0.20
iloprost	0.82 ± 0.12[a]	11.40 ± 0.20[c]	0.39	0.39
PGI_2	0.66[e]	2.60[e]		

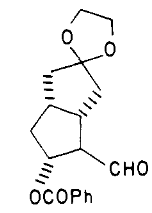
[a] Four different experiments. [b] Four experiments with four different prostacyclin concentrations each. [c] The IC_{50} values for ZK 96 480 and iloprost were significantly different with $p \leq 0.01$. [d] Two experiments only. [e] One experiment only.

-20 °C) in the presence of *N*-bromosuccinimide furnished the α,β-unsaturated bromo ketone **10** in 60% yield: oil; ^{1}H NMR (CD_2Cl_2) δ 1.04 (3 H, t, *J* = 7.5 Hz, CH_2CH_3), 1.14 (3 H, d, *J* = 7 Hz, $CHCH_3$), 3.91 (4 H, m, OCH_2CH_2O), 5.21 (1 H, m, H-11β), 7.09 (1 H, d, *J* = 10 Hz, H-13), 7.42–7.92 (5 H, m, COPh); IR (neat) 1720 (COPh), 1690 (COC=C) cm^{-1}. Reduction of **10** ($NaBH_4$, CH_3OH, -40 °C) gave a ca. 1:1 mixture of the allylic alcohols **11a** and **11b**, which was separated chromatographically.[10] Dehydrobromination (50% aqueous NaOH, toluene, catalytic NBu_4/HSO_4, 25 °C) of the less polar alcohol **11a** with concomitant saponification of the benzoate group followed by acidic (HOAc, H_2O) cleavage of the ketal moiety afforded the ketone **12** (73% from **11a**): oil; ^{1}H NMR (CD_2Cl_2) δ 1.06 (3 H, d, *J* = 6.8 Hz, $CHCH_3$), 1.10 (3 H, t, *J* = 7.5 Hz, CH_2CH_3), 4.22 (1 H, m, H-11β), 4.38 (1 H, m, H-15β); IR (neat) 1730 (C=O) cm^{-1}. After silylation of the hydroxyl groups in **12** ($ClSiMe_2$-*t*-Bu, DMF, imidazole), the ketone **13** was subjected to a Horner–Wittig reaction with triethyl phosphonoacetate (KO-*t*-Bu, THF, 0 °C). Reduction of the 1:1 mixture of the isomeric α,β-unsaturated esters **14** with diisobutylaluminum hydride (toluene, 0 °C) gave after chromatographic separation the *E* isomer **15a** (32% from **12**) and the less polar *Z* isomer **15b**.[11]

Etherification of **15a** under phase-transfer conditions with *tert*-butyl bromoacetate (50% aqueous NaOH, toluene, catalytic Bu_4NHSO_4, 25 °C) was accompanied by simultaneous cleavage of the *tert*-butyl ester to give **16** (87%). Finally, removal of the silyl ether groups (tetra-*n*-butylammonium fluoride, THF, 25 °C) afforded **2** in 86% yield: oil; ^{1}H NMR (CD_2Cl_2) δ 1.07 (3 H, d, *J* = 6.8 Hz), 16β-CH_3), 1.11 (3 H, t, *J* = 7.5 Hz, CH_2CH_3), 3.97 (1 H, m, H-11β), 4.06 (2 H, m, OCH_2CO), 4.12 (2 H, m, =$CHCH_2O$), 4.37 (1 H, dd, *J* = 5.5, 1.0 Hz, H-15β), 5.51 (1 H, m, H-5); IR (neat) 1730 (COOH) cm^{-1}.

Compound **2** is a potent inhibitor of platelet aggregation in human and rat PRP (platelet rich plasma) (Table I) and was shown to have practically the same affinity to the prostacyclin receptor as PGI_2 and iloprost with use of a particulate fraction of human platelets.[12]

The antiaggregatory potency was also tested in vivo in anesthetized rats by inducing intravascular platelet aggregation[13] by intravenous infusion of 30 μg/kg per min



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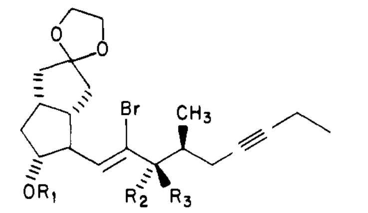
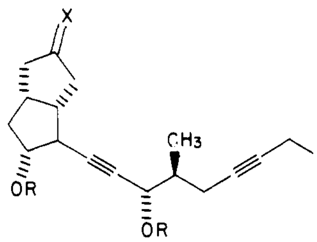
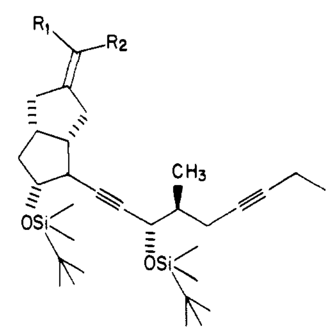


10, R_1 = COPh; R_2 = R_3 = O
11a, R_1 = COPh; R_2 = OH; R_3 = H
11b, R_1 = COPh; R_2 = H; R_3 = OH



12, X = O; R = H
13, X = O; R = $SiMe_2$-*t*-Bu
14, X = CHCOOEt; R = $SiMe_2$-*t*-Bu



15a, R_1 = CH_2OH; R_2 = H
15b, R_1 = H; R_2 = CH_2OH
16, R_1 = CH_2OCH_2COOH; R_2 = H

ADP lasting for 2.5 min and was compared with the hypotensive effects of **2** in the same species. In these experiments **2** mimics the pharmacological profile of iloprost, the threshold dose for in vivo platelet aggregation inhibition being approximately 5 times lower than the threshold dose for the hypotensive effect.

On oral application of **2** (0.01–1.0 mg/kg), the blood pressure of SHR (*n* = four to six animals/dose group) decreases rapidly with a threshold dose of 0.01 mg/kg (iloprost >0.05 mg/kg). The maximum reduction of mean arterial blood pressure to 63.7 ± 1.4% ($\bar{x}$ ± SEM) of the initial value is obtained with a dose of 0.1 mg/kg (iloprost 0.5–1 mg/kg). The heart rate increases in a dose-dependent manner, reaching 156.5 ± 6.55% ($\bar{x}$ ± SEM) of the initial value with 1.0 mg/kg of **2**.

With respect to threshold doses and maximum effective doses, **2** is at least 5 times more effective than iloprost. Most importantly, the hypotensive action after oral application of 0.1 and 0.5 mg/kg of **2** lasted 2–3 times longer than that of 5 mg/kg iloprost.[14]

Acknowledgment. We thank Marion Slopianka, Klaus Cornelius, and Detlef Schmidt for their excellent technical assistance and Dr. A. Seeger for the interpretation of the NMR data.

Registry No. **2**, 94079-80-8; (±)-**3**, 99783-70-7; (±)-**4**, 99783-71-8; **5** (isomer 1), 99783-72-9; **5** (isomer 2), 99783-73-0; **6**, 99828-08-7; **7**, 99783-74-1; **8**, 99783-75-2; **9**, 74818-14-7; **10**, 99828-09-8; **11a**, 99828-10-1; **11b**, 99828-11-2; **12**, 95639-59-1; **13**, 99783-76-3; (*E*)-**14**, 99783-77-4; (*Z*)-**14**, 99828-12-3; **15a**, 99783-78-5; **15b**, 99828-13-4; **16**, 99783-79-6; D-(-)-$PhCH(NH_2)CH_2OH$, 20989-17-7; $(CH_3O)_2P(O)CH_2Li$, 73778-54-8; $(C_2H_5O)_2P(O)CH_2CO_2C_2H_5$, 867-13-0; $BrCH_2CO_2C(CH_3)_3$, 5292-43-3; $C_2H_5O_2CCH(CH_3)CO_2C_2H_5$, 609-08-5; $BrCH_2C{\equiv}CCH_2CH_3$, 16400-32-1; C_2-

(10) The less polar fraction on TLC was assigned the structure of the 15*S*-isomer **11a** and the more polar one as the 15*R*, based on the known chromatographic behavior of synthetic PG intermediates.

(11) The configuration of the trisubstituted Δ^5-double bond is established by comparison of the biological activities of the target compound **2** and the corresponding unnaturally configurated *Z* isomer.

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$H_5C{\equiv}CCH_2C(CH_3)(CO_2C_2H_5)_2$, 83067-48-5; $C_2H_5C{\equiv}CCH_2C$-$H(CH_3)CO_2C_2H_5$, 99783-80-9.

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Unsaturated Heterocyclic Amines as Potent Time-Dependent Inhibitors of Dopamine β-Hydroxylase

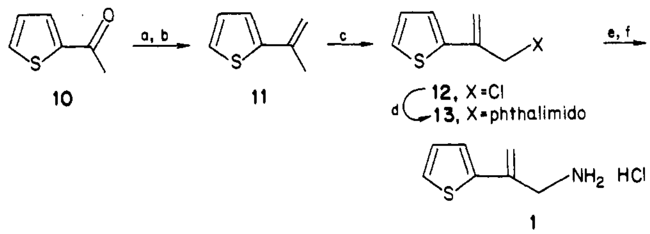
Sir:

Dopamine β-hydroxylase (DBH; EC 1.14.17.1), a copper-dependent monooxygenase, catalyzes the conversion of dopamine to norepinephrine in the peripheral sympathetic as well as in the central nervous systems.[1] The enzyme is easily inhibited by copper chelators, but these types of inhibitors lack selectivity; the most notable example is fusaric acid, which was studied in the clinic for the treatment of hypertension.[2,3] Recently, a number of enzyme-activated inhibitors of DBH have been reported in the literature: β-chlorophenethylamine,[4] 4-hydroxybenzyl cyanide,[5] 2-halo-3-(*p*-hydroxyphenyl)-1-propenes,[6] 1-phenyl-1-propyne (**9**),[7] and 2-phenylallylamine (**8**).[8] Despite their progressive increases in activity as time-dependent inhibitors, the most effective of these compounds remains in the millimolar potency range and none have been reported to exhibit antihypertensive activity.

We report that, contrary to previous belief,[9] certain heteroaromatic amines can serve as substrates[10] and as exceptionally potent time-dependent inhibitors of dopamine β-hydroxylase. Indeed, 2-(2-thienyl)allylamine hydrochloride (**1**) exhibited a greater than 1000-fold enhancement in activity over the corresponding phenyl analogue (**8**)[8] and has antihypertensive activity in the spontaneously hypertensive rat (SHR). In addition, we report that 3-phenylpropargylamine (**7**) is equipotent to **1** as a time-dependent inhibitor of DBH.

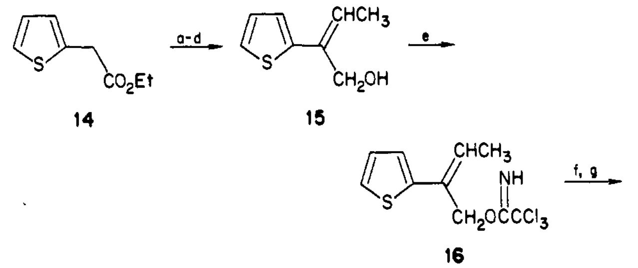
Chemistry.[11] 2-(2-Thienyl)allylamine (**1**) was prepared as outlined in Scheme I. 2-Acetylthiophene (**10**) was allowed to react with methylmagnesium bromide and the resulting alcohol dehydrated to 2-isopropylidenethiophene (**11**). The allylic chlorination procedure of Hori and Sharpless[12] was used to provide a mixture of allyl chloride **12** and vinyl chlorides, which was immediately allowed to react with potassium phthalimide. Deprotection of the highly crystalline phthalimide **13** provided the desired amine **1**. The 3-thienyl regioisomer **4** and the 2- and 3-furanylallylamines **5** and **6** were prepared by the same reaction sequences with the exception that the synthesis of **6** started with ethyl 3-furoate. This starting material was converted to the corresponding tertiary alcohol with 2 equiv of methylmagnesium bromide and dehydrated as in the preparation of **11**. *N*-Methylallylamine **3** was prepared by reaction of *N*-methyltrifluoracetamide with purified allyl chloride **12** (NaH/DMF/80 °C) and hydrolysis of the resulting allyl trifluoacetamide during workup (1 N NaOH).

Scheme I[a]



[a] (a) CH_3MgBr, (b) $KHSO_4$, (c) NCS, $(PhSe)_2$ (cat.), pyr (cat.), (d) potassium phthalimide/DMF, 90 °C, (e) $NH_2NH_2{\cdot}H_2O$/EtOH, (f) HCl/Et_2O.

Scheme II[a]



[a] (a) LDA/THF/–70 °C, then CH_3CHO, (b) $MsCl/Et_3N/CH_2Cl_2$/20 °C, (c) DBU/CH_2Cl_2/20 °C, (d) 2 *i*-Bu_2AlH/CH_2Cl_2/–70 °C, (e) CCl_3CN/CH_2Cl_2/DBU (cat.)/20 °C, (f) xylene, reflux, (g) KOH/EtOH/40 °C.

α-Methylallylamine **2** was synthesized according to Scheme II. 2-Thiopheneacetic acid ethyl ester **14** was deprotonated and the resulting enolate was trapped with acetaldehyde to furnish a mixture of diastereomeric alcohols. The crude alcohols were converted to a geometric mixture of olefin esters which were reduced to the corresponding allyl alcohols **15** with diisobutylaluminum hydride. Alcohols **15** were treated with trichloroacetonitrile in the presence of a catalytic quantity of DBU to furnish trichloroacetimidates **16**. Rearrangement was effected by using Overman's methodology[13] to provide the corresponding allyltrichloroacetamide. Base-promoted hydrolysis yielded the desired α-methylallylamine **2**.

3-Phenylpropargylamine (**7**) was prepared by the literature procedure.[14]

Biochemistry and Pharmacology. DBH was purified from beef adrenals following a described procedure.[15] The enzyme was homogeneous in SDS gel electrophoresis and

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(10) The 2- and 3-substituted thiophene and furan ethylamines serve as substrates for DBH with V_{max}/K_m values in the range of that for tyramine. These results will be discussed in the full paper.
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